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Vol. XXXVI. *April, 1919.* *No. 4*

BIOLOGICAL BULLETIN

PRECIPITATION-STRUCTURES SIMULATING ORGANIC GROWTH. II.

A Contribution to the Physico-Chemical Analysis of Growth and Heredity.

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Part I. Theoretical.

The problem of growth, reduced to its simplest terms, is the problem of the conditions under which structure of a definite and specific kind is built up by the growing system through the chemical and physical transformation of material taken from the surroundings. As thus expressed, our definition applies to inorganic as well as to organic growth, *e. g.*, to the formation of a crystal from its "mother-liquid," or of a metallic deposit at a cathode. Both of these processes, especially the latter, exhibit many significant analogies to organic growth-processes; thus a crystalline or electrolytic deposit of a given chemical composition, laid down under constant external conditions, has, like an organic growth, its own definite and specific structural peculiarities. In organic growth and development, however, numerous complexities enter which are absent from inorganic growth; in particular the continual chemical and physical activity of the living system is always present as a dominating factor; this activity is itself specific and modifies in a specific manner the structure-forming processes, and is itself modified by them. Since every living organism is by its very nature an active system of this kind, the problem of organic growth becomes one relating not merely to the origination of specific *structure* but of specific physiological processes and activities as well, some of which are demonstrably dependent upon the observed structure, while others are related

to it in a manner which cannot be precisely defined at present. Evidently structure, as such, in the sense of fixed disposition of material parts, represents only one side of the vital organization; its other and more characteristic side is manifested in the various special organic processes and the external behavior of the organism; these in turn imply the regulated concurrence, interaction and sequence of numerous simpler processes and events of a purely physico-chemical nature.

It is necessary, therefore, in forming a conception of the vital organization, to include as elements *all* of those constant or regularly recurrent features, both of structure and of activity, which contribute to the persistence or stability of the organism, considered as an individualized part of nature—*i. e.*, as a system in equilibrium with an environment. Any living being represents an integration or unification not merely of static conditions like structure, but also of *processes*, both simultaneous and successive; *i. e.*, the organism is to be considered not simply as a physical whole which is complete at any given moment, but rather as in its essential nature an ordered or regulated *sequence* of changes and activities, coördinated in space and time. For example, the succession of formative and physiological processes constituting development, or any special instinct requiring time for its exercise, is just as constant and characteristic a feature of the organization of a species as is its skeletal structure. What is most remarkable in the organism is the exact coördination of the various single activities, widely separated as they often are both spatially and temporally. Simultaneity in certain processes, ordered succession in others, are equally essential to its stable working; to be concrete, the normal activity of the heart and circulation in higher animals requires the simultaneous and coördinated activity of the nervous system, and the process of digestion depends upon the constancy of a certain definite succession of events in the alimentary tract and its related organs. All of this must be remembered in considering the problem of organic growth; we have to account for the construction of a system which is complex and specific in its *activities*, as well as in its *structure*.

It will be agreed that the possibility of any special organic

function, consisting, as it typically does, in a definite and regular succession of simpler processes, depends upon the presence of a fixed and stable structural organization; without this there can be no organization of processes, *i. e.*, physiological organization, any more than there can be organized or coördinated activity without coördinated structure in an artificial mechanism like a locomotive. In this sense structure is of primary importance in any organism; the distinctive features of vital organization must therefore be referred ultimately to special structural peculiarities. This is obviously true of the various physical mechanisms and devices subserving particular functions (like locomotion, vision, circulation of blood, etc.) in higher animals; but it is the less familiar application of this principle to the general conditions of organic growth that I wish to consider in the present paper.

The structural conditions present at a given time in a growing or developing organism determine its present and future possibilities of growth. In a certain sense this is already well recognized; a definite structure can be observed in many instances in the germ before development, and certain features of this structure, especially the symmetry about certain axes and the distribution of certain materials, foreshadow the general morphological plan of the adult organism.[1] But in its details the structure of the egg is entirely different from that of the adult, and its precise relation to the latter must be regarded as unknown; *i. e.*, we cannot say definitely why in any species the transformation of the germ and of the material which it incorporates from the surroundings follows a constant and definite course, leading to a definite final or adult stage. Since, however, the building-up of organic structure from food and other materials is obviously a matter of constructive metabolism, any physicochemical theory of development must assume that a main factor in the formative process is the influence of the structural conditions already present upon the metabolic transformations by

[1] For a general account of the known correspondences between the structure of the egg and that of the adult, *cf.* Conklin's recent book, "Heredity and Environment in the Development of Man," Princeton University Press, 1918; also his recent paper, "The Share of Egg and Sperm in Heredity," *Proc. Nat. Acad. Sci.*, 1917, Vol. 3, p. 101.

which more structure is formed.[1] At each stage of development the structure already present determines the nature of the structure which is being formed at the time; *i. e.*, the formative metabolism, like metabolism in general, is controlled by the structural conditions present in the growing regions, at the site of the syntheses and other chemical reactions concerned in the constructive process.[2] In considering the general physiology of growth the first question to be asked relates to the manner in which structure of a certain kind determines the production of further structure of the same or similar kind. Differentiation in ontogeny presents certain additional problems, but all developmental processes presuppose *growth*, or increase in organized material of a type already present.[3]

It is to be assumed that the chemical reactions constituting the specific metabolism of any cell or other living system are under the control of structural conditions of a highly definite kind. Protoplasm is polyphasic in its physico-chemical constitution, and both the nature and the velocity of the chemical reactions in such a system must be largely influenced by boundary conditions (adsorption, capillarity, interfacial potentials, etc.); hence in a system of a definite chemical composition constancy in the arrangement, character and extent of the phase-boundaries, *i. e.*, in the *structural* conditions present in the reacting system,

[1] The general features of this relation are very clearly discussed in Child's "Senescence and Rejuvenescence," University of Chicago Press, 1915, Chapter 1, pp. 26 *seq.*; *cf.* also the special papers there cited.

[2] It should be noted that the site of the structure-producing chemical changes may in some cases be *extracellular;* materials produced by normal metabolism within the cell may pass to the exterior (be secreted, etc.) and later undergo chemical change giving rise to definite structure; supporting or skeletal structures are often thus formed (matrix of bone, cartilage, connective tissue, etc.); other instances are formation of spiders' webs and similar structures, clotting of blood, etc.

[3] In differentiation, *i. e.*, the general process by which groups of descendants of the same germ-cell become structurally and physiologically different and functionally specialized in later development, factors seem to enter which are absent in simple growth. The phenomena of Mendelian inheritance plainly suggest that differentiation is controlled by the distribution of a limited number of structure-determining factors embodied in chemically stable material particles which can be transferred with properties intact from cell to cell. The chromosomes answer to this conception, and apparently form an essential part of the special physiological mechanism controlling differentiation; of course this control must be exercised by modifying metabolism, in a manner and by means which are as yet unknown.

implies corresponding constancy in the reactions taking place under such influence. In other words, if the structural conditions are definite, the chemical processes whose rate, nature and position they control must also be definite; and the products of these reactions, if they are of such a nature as to accumulate and add to the structural material of the system, will also be definite and will be deposited in definite situations and at definite rates and times. Apparently deposition of this kind forms an essential part of the organic growth-process.[1] Any structural material thus added will secondarily influence further growth, and by degrees, if metabolic activity continues, a definite structure will be built up whose precise nature, when the final stage is reached, will have been determined by the structure originally present in the growing system—assuming constancy in the original chemical composition of the latter, and in the conditions to which it has been exposed during its growth.

In any living organism the characteristic physiological activities and external behavior must have as their basis a constancy and specificity in the chemical and physical conditions underlying the fundamental vital processes. These conditions are in large part structural; if we could account fully for specific structure, we could not doubt account also for what is specific in physiological activity and behavior. Now specific structure is not confined to organisms; it is perhaps most perfectly exemplified in the specific form of crystals; and crystalline form is to be referred in turn to the chemical character and mode of arrange- of the molecules composing the crystal. In attempting to account for the specific form-characters of organisms we must first inquire, as a matter of general scientific procedure, whether it is not possible to refer these to the more general conditions present in other natural systems which also exhibit definite and specific form-characters. The case of crystals is the clearest; here the visible structure is related to the structure of the molecules and varies with changes in the nature and relative positions of the atoms; this relation of molecular configuration to crystalline form is best illustrated by the differences in the right and left-handed crystals of stereoisomeric compounds like the tar-

[1] Compare the interesting discussion of Child, *loc. cit.*, Chapter 2, pp. 38 *seq.*

trates. In the chief compounds forming the structural basis of living protoplasm, the proteins, we have molecules of great complexity, consisting essentially of chains of asymmetric molecules united by an easily dissoluble linkage; it is this structure which renders possible the high degree of specificity shown by the individual proteins. There is definite evidence that to this chemical specificity corresponds an equally definite morphological or structural specificity in the crystalline or other aggregates formed by proteins when they separate out from solution. Reichert and Brown's observations indicate that if our knowledge were sufficiently exact and detailed different species could be distinguished and identified by the crystal-forms of their hæmoglobins,[1] just as birds can be distinguished by their feathers; and presumably similar relations between specific molecular structure and the structure of solid aggregates hold true for homologous proteins of other kinds. Probably it is to the intimately or microscopically crystalline nature of the deposits that the possibility of distinguishing different protein precipitates by their microscopic characters is due.[2] According

[1] *Cf.* Reichert's very suggestive article, "the Germ-plasm as a Stereochemic System," *Science*, 1914, N.S., Vol. 40, p. 649. Reichert also regards heredity as ultimately an expression of the influence of the specific structural characters of the cell-proteins upon the course of formative metabolism. "Given as the basis of scientific study a germ-plasm that has inherently the power of development; that is in the form of a stereochemic system that is peculiar to the organism; that is highly impressionable to stimuli; and that has the marked plasticity that is inherent to organic colloidal matter, we have all the postulates that are needed as a foundation upon which, according to the laws of physical chemistry, can be built a logical explanation of the essential fundamental elements of the mechanism of heredity . . . The typical condition of matter of definite composition is crystalline. . . . Having a homogeneous solution of various selected crystalline substances of appropriate chemical composition and constitution, and given conditions attendant to crystallization, the successive stages of crystalline development will proceed along fixed and definitely recognized lines. . . . Having in the germ-plasm an analogous physico-chemical system, . . . the phenomena of development likewise proceed in conformity with the same laws along definite lines . . . " (pp. 656–7). These quotations will indicate Reichert's general point of view. His conception of the manner in which this specific structure influences metabolism and thus determines further structure-formation is, however, different from that outlined in the present paper.

[2] This seems possible to a considerable degree. Alfred Fischer's work, "Fixierung, Färbung und Bau des Protoplasmas," Jena, 1899, Chapters 3–5, represents a beginning in this direction; further development of this technique seems desirable.

to von Weimarn all solid material, including matter in the "amorphous" and colloidal states, is ultimately crystalline in structure;[1] and this general conception is in conformity with the more recent evidence from other departments of physics indicating that the molecules in solid matter are oriented regularly to form definite geometrical patterns (space-lattices).[2] If therefore to a specific molecular structure there corresponds a specific *molar* structure (or structure peculiar to the substance in its solid state of aggregation), it follows that any specific protein which is synthesized and laid down in solid form within the cell, or at the cell-surface or in any other situation, will necessarily produce a definite and specific type of structure different from that formed by any other protein. Such a chemical synthesis would thus involve a morphological synthesis of a simple kind; to the constant structure of the single molecules would correspond certain constant form-characters in the larger aggregates built up from these molecules. It seems clear, therefore, that if specific proteins are synthesized in any organism, and if

Della Valle's view that the form-characters of individual chromosomes are an index of a definite microcrystalline structure is especially interesting, since in this case a specific chemical composition of each chromosome would be implied by its specific and constant form-characters. *Cf.* Della Valle: *Archivio Zoologico Italiano*, 1912, Vol. 6, p. 37; also *Zeitschr. f. Chemie u. Industrie d. Kolloide*, 1913, Vol. 12, p. 12.

[1] Von Weimarn's observations and theoretical discussions are chiefly published in *Zeitschrift für Chemie und Industrie der Kolloide*, 1907–13, Vols. 2—13, though partly in Russian journals. The work is too extensive to be summarized briefly, but its experimental part consists largely in showing that all transitions between typically colloidal systems of inorganic and organic compounds and definitely crystalline systems may be obtained by varying the conditions of formation of the compounds, *e. g.*, the concentrations of the solutions (*e. g.*, $Ba(CNS)_2$ and $MnSO_4$) mixed to form the compound in question, the solubility of the latter, its rate of formation, etc. In this manner the crystalline structure of gels and other colloidal systems is clearly indicated. Protein gels are thus crystalline in the microscopic or submicroscopic character of their solid phase, and hence possess a specific inner structure. The crystalline nature of fibrin gels has recently been clearly shown by Howell; *cf. Amer. Journ. Physiol.*, 1914, Vol. 35, p. 143, and 1916, Vol. 40, p. 526. For the microcrystalline structure of inorganic precipitation-membranes *cf.* von Weimarn, *loc. cit.*, 1908, Vol. 2, pp. 280 *seq.*, and photographs there reproduced.

[2] *Cf.* the papers by W. H. and W. L. Bragg on the patterns produced by the diffraction of X-rays at the surfaces of crystals, indicating a space-lattice arrangement of the atoms: *Proc. Roy. Soc.*, 1912, Vol. 88 A, p. 428, and 1913, Vol. 89 A, pp. 248, 277, 430, 468.

their molecules segregate and the compounds separate out in solid form, specific structure will be the inevitable result. And this structure, having once arisen, will exert its own specific influence upon the ensuing chemical and physical transformations in the system. Any metabolism thus controlled by specific structure must exhibit specific features, and if it produces further structure this will also be specific, and will influence specifically the succeeding metabolic processes by which more structure is formed.[1]

If we consider the growth and development of the germ in the light of these general considerations, we are led irresistibly to the conclusion that in any specific sequence of form-changes, such as those constituting development in any organic species, the character of the formative transformation proceeding at any stage, as well as the special character of the terminal stage of the whole sequence, the adult organism, must be determined by the original structure of the system—that with which the growth begins. It is clear that the conditions, structural and other, present when growth is *begun* must affect the entire succeeding series of transformations. The initial factors, however, are not the only ones to be considered; changes in external conditions appearing at any time during the series must contribute their influence; and the eventual outcome will be determined not only by the initial state of the growing system and its surroundings, but also by the conditions present at any time during the process of growth. This conclusion clearly implies that if the surrounding conditions are kept *constant*, a growing system of a given initial constitution will undergo a series of transformations

[1] Obviously any chemical synthesis involves a morphological synthesis, in the sense of producing molecules with a definite spatial distribution of atoms and hence a definite geometric form or configuration. But it is only when these molecules unite or segregate (with axes parallel) to form solid structures like crystals or crystal-aggregates, that the chemical synthesis becomes the condition of morphological synthesis in the crystallographic sense; the vectorially acting interatomic forces determine the special configuration of the molecule, and when union occurs to form large aggregates the latter exhibit specific geometrical characters which are determined by those of the individual molecules. The present contention is simply that morphological synthesis in organisms is also ultimately determined, in the somewhat indirect manner indicated above, by this structural specificity of the solid material produced in metabolism. A definite internal structure is thus imparted to protoplasm, and this determines a definite and specific formative as well as other metabolism.

which will also be constant, *i. e.*, definite or predetermined. The whole developmental sequence may then be said to have been *determined* by the original constitution of the system; that is to say, two or more such similar systems, placed under the same external conditions, will develop in the same way and will reach similar final stages at the same time. Conversely, two systems *differing* in initial constitution, placed under similar external conditions, will be transformed in different manners and at different rates and will form different final products. The case of two eggs of the *same* species developing side by side in sea-water illustrates the first case; that of two eggs of *different* species the second. The corollary follows, that any attempt to account causally for the special structure or activities of an organism at any stage, adult or embryonic, must always involve a *regressus* to the original conditions with which development starts. One is thus inevitably brought back to the organization of the germ as a starting point.[1]

The general conclusion follows: any germ with a definite or fixed physico-chemical organization, implying constancy in the composition, distribution, and physical state of its structural and other chemical components, which is in process of incorporating materials from the surroundings (or its own yolk reserves) and transforming these materials so as to form further structure, will necessarily follow a constant course of transformation if the external conditions are also constant; this will apply both to the type of structure making its appearance at any stage, and to the physiological processes and other activities whose nature depends on that structure. Apparently we have here the general type of situation presented by the developing germ or other living system in process of growth. Given a specific organization at the outset, together with constancy of conditions, external and internal, under which the incorporated

[1] The origin of this organization is of course similarly to be referred to preëxisting conditions, and strictly speaking any such *regressus* is *ad infinitum*. In any sequence of physical processes each stage is at once conditioned by the preceding and the condition of succeeding stages—*i. e.*, is both determined and determinant. For an admirable discussion of the relation of this feature of reality to developmental processes in general, *cf.* Hobhouse's "Development and Purpose," Part 2, especially chapters 4 and 5.

material is transformed and accumulated, and the system will undergo a cycle of chemical and physical transformation which will itself be constant and specific. That is, two or more such systems will undergo identical transformations. This, put simply, appears to be the general property or condition which in living organisms we call "heredity."

The artificial growing systems considered in the present and preceding papers exhibit in a simplified form the same general type of behavior. We have seen that in a system of a given composition chemical reactions occurring under structural conditions of a definite kind will give rise to definite reaction-products at a definite rate and in definite situations; hence if solid—*i. e.*, structurally coherent and stable—material is among these reaction-products, this material as it accumulates will add to the already existing structure in the system and will itself influence in a definite manner the succeeding reactions and structure-formation. In the formation of precipitation-structures or crystallization-structures by combinations of mutually precipitating dissolved salts this general type of phenomenon is exemplified in a simple manner; and when the precipitates have certain physical properties—low solubility, fine subdivision, colloidal quality, coherence—the structures thus formed often exhibit a close resemblance to simple types of organic growth. Precipitation-structures of copper ferrocyanide, made by running a solution of copper sulphate beneath a solution of potassium ferrocyanide, have long been used to illustrate this phenomenon. The essential basis for this resemblance is undoubtedly the formation of semi-permeable membranes by the precipitate as it forms; these, by preventing diffusion, furnish the conditions for the osmotic entrance of water into the various membrane-enclosed spaces (which are typically of a vesicular, cellular or tubular form); a more or less definite localization of osmotic processes and of the direction of flow of solution results; hence the deposition of precipitate is also localized and a correspondingly definite structure is formed, which resembles that of simple vegetative growths because of the importance of the osmotic factor in both cases. The size, shape, and other characters of the structures formed under these simple conditions are not so

constant as in the majority of organic growths; but much deviation from type is to be expected when accidental variables are not compensated or excluded—as they typically are in the delicately regulated growth-processes of living organisms. Even in organisms, however, the degree of form-regulation varies, and in many plants especially the "habit" of growth is not fixed but changes with the conditions. Precipitation-structures formed according to the directions of Leduc or Herrera from a prescribed combination of materials[1] exhibit many morphological features which are constant and determined mainly by the chemical nature of the structural material. Similarly in the precipitation-structures formed from metals by electrolytic local action, as described in the present and preceding papers, the tubules and vesicles of *iron* ferricyanide are characteristically different in appearance and structure from those of (*e. g.*) *zinc* ferricyanide formed under the same external conditions; in fact each metal (Fe, Co, Ni, Zn, Cd, Cu) forms a specific and easily recognizable type of precipitation-structure. The chemical nature of the structural material—insoluble precipitate of inorganic salt in this case—determines the special form-characters assumed by the single filaments, vesicles and other structures which grow out from the metal; and if these structures are allowed to accumulate until conditions of equilibrium are reached and growth ceases, the whole resulting mass of precipitation-formation is then found to present an appearance of a constant and characteristic kind (see photographs, Plates 1–6).

In the analogous case of the living organism, *e. g.*, a higher animal, where a definite and final form and structure are attained at the end of a long period of development and growth, it is known that the structural material has a constant and highly specific chemical composition; and it is to be assumed that this composition has a determinative relation to the physical and other properties of the organized structure thus arising. In this respect the two types of growing system under comparison exhibit certain fundamental similarities in the general physico-chemical nature of the formative process; and it seems desirable

[1] *Cf.* Leduc, "Mechanism of Life," London, Rebman Ltd., 1911; Herrera, *Boletin de la Direcion de Estudios Biologicos, Mexico*, Vols. 1 and 2, 1916 and 1917.

to recognize these resemblances and to formulate them clearly (without of course ignoring or underestimating the many and obvious differences). In both the living and the non-living growing systems the structural material is the product of chemical reactions whose rate, character, and position depend upon the chemical nature of the interacting substances and upon the structural conditions under which the reactions take place. In the living system, however, on account of the minutely graded chemical and structural specificity and the exact regulation of the conditions under which the metabolic and structural transformations occur, the constancy or determinateness of form, structure, and activity when the final or adult stage is reached is much greater than in the inorganic system; and it is only in its broadest and most general features that the growth of the latter can be regarded as a model for that of a living organism. Indeed the most astonishing feature of organisms has always been that from a minute germ a system of the utmost chemical and physical complexity, yet identical in all respects with another system arising similarly from another germ of the same kind, should be built up by the chemical transformation of materials taken from the surroundings. Nevertheless something of the same kind is seen in the building-up of a precipitation-structure under the influence of a piece of metal placed in a ferricyanide solution. Here also the material of metal and solution is chemically and structurally transformed in a constant manner, and a system of definite physico-chemical constitution and activity is formed whose peculiarities of structure and activity have a specific dependence upon the nature of the introduced metal.

The precise relation between the specific chemical constitution of the proteins of the living germ—*i. e.*, of those structural compounds which are demonstrably peculiar to the species in question—and the specific type of structure arising in growth or development is unknown. It must be recognized that other factors than the chemical specificity and arrangement of the structural proteins play a part in determining the precise course of the formative process; *e. g.*, changing the nature and proportions of the salts of sea-water produces definite modifications of development in the eggs of marine animals; well-known examples

of this effect are the cyclopia induced in fishes by adding magnesium salts,[1] and the production of exogastrulæ in sea-urchin eggs by lithium chloride.[2] The germinal protoplasm contains inorganic salts and numerous other non-specific compounds, differing in their nature, concentration, and distribution in different germs; moreover the catalysers present and the various purely physical peculiarities of the system (*e. g.*, viscosity, capillarity, boundary-potentials, permeability to diffusing substances, etc.) must all enter as factors influencing the rate and character of the chemical and physical processes concerned in development. All such factors must be included under the conception of *germinal constitution;* but we are still far from understanding why a given germinal constitution determines in such minute detail the course of the proliferative and transformative process constituting development in any particular species. It seems certain, however, that chemical specificity is the primary and fundamental factor. All organic structure is built up by chemical processes, *i. e.*, by metabolism; hence the divergences first appearing in evolution between different species must have been primarily *chemical* in nature, since they obviously involved—or consisted in—divergences in the character of the specific metabolic syntheses on which the growth, continued life and special character of any organism depend. The essential differences in the germinal constitutions of different species must similarly depend on specific chemical differences, *i. e.*, primarily on the specific constitution of the proteins; these differences will determine specific structural differences, as we have seen. For example, it seems probable that the egg of the starfish and that of the sand-dollar—which are closely alike in general appearance and properties—are not widely dissimilar in their purely chemical constitution, except as regards the specific nature of their structural proteins; and that it is ultimately because of this difference that the two germs effect such different transformations in the food and other materials which each incorporates from the surroundings and builds up into an adult organism of constant type.

[1] Stockard, *Archiv f. Entwicklungsmech.*, 1907, Vol. 23, p. 249; *Journ. Exper. Zoöl.*, 1909, Vol. 6, p. 285.

[2] Herbst, *Zeitschr. wiss. Zoöl.*, 1892, Vol. 4, p. 446; *Mitt. zoöl. Sta. Neapel*, 1893, Vol. 11, p. 136.

But a detailed knowledge of the constitution of these proteins, *i. e.*, of the chemical nature and serial (or other) arrangement of the amino-acids united to form the protein molecule, would not at present—if ever—enable us to predict the course of development of either egg.

We may assume, however, that the essential reason for such a ailure would be that the conditions in the living system are too *complex* (for historical and other reasons) to be analyzable in the requisite detail, and not that a sufficiently complete knowledge of the initial state of the system would never enable us to predict the general course and outcome of the developmental transformation. Under these circumstances the study of some relatively *simple* inorganic process, which in certain respects may serve as a model for the organic formative process, may throw light upon the general features of such a problem and indicate the type of solution which it is practicable to seek. The purely scientific aim is not to follow the developmental sequence through all of its stages and account causally for each separate transformation, but rather to determine what the essential and constantly operative factors are in *all* cases of development,—*i. e.*, the *general* conditions which make possible a specific type of development in any particular case. Each case presents its own special problems; but there are factors common to all cases of development, and any general theory of development requires that the nature of these should first of all be clearly indicated.

In the inorganic growth-structures described in the present and preceding papers[1] certain definite relations can be recognized between the type of structure formed and the chemical and physical character of the precipitate which is deposited to form that structure. Thus filaments of zinc ferricyanide are coarser and less coherent, as well as more variable and irregular in form, than filaments of iron ferricyanide formed in the same solution (see Figs. 1–5, 12–15). The metal may be compared with the germ; its specific chemical nature determines the special type of structure to which it gives rise in a given solution of potassium ferricyanide—*i. e.*, under definite external conditions; the reason for this is that each insoluble ferricyanide has its

[1] R. S. Lillie, BIOL. BULL., 1917, Vol. 33, p. 135.

own special physical and structural peculiarities which vary with the nature of the heavy metal. The observed structural difference between these two kinds of filaments appears to be largely determined by the difference in the physical nature and consistency of the precipitate; this is more finely divided and coherent with iron than with zinc, and greater smoothness of contour and uniformity of structure in the growing filaments are the result. Again, the two closely related metals, nickel and cobalt, whose ferricyanides have almost identical physical properties, illustrate the same principle in a highly characteristic manner; the precipitation-structures formed from these two metals are strikingly similar in appearance, exhibiting what might be described as a close family resemblance, *e. g.*, in the coarse texture and opacity of the filaments and in the characteristically winding and tortuous course which they both adopt (see Figs. 16, 17, 21); this latter peculiarity is found in none of the filaments formed from other metals. Similarly zinc and cadmium form filaments and vesicular formations (Figs. 12, 13, 18, 19) which are closely alike in structure and appearance.

It is interesting to note that this resemblance extends not only to the form-characters of the completed structure but also to the peculiar type of physical activity which it exhibits during its growth. The precipitate forming the walls of the striated vesicular or shell-like structures characteristic of zinc and cadmium (see Figs. 13, 18, 19) is deposited rhythmically or intermittently; the striation is a direct consequence of this rhythm, which itself is due to an intermittency in the outflow of the solution from the interior of the growing vesicle; this intermittency depends upon the alternating formation and rupture of the precipitation-membrane forming the wall of the vesicle. The rate of this rhythm, and hence the rate at which the precipitate is deposited and the resultant character of the striation, are similar in the two metals. Evidently these peculiarities of behavior are to be referred ultimately to the special physico-chemical properties of the precipitate and of the metal; zinc and cadmium, being chemically closely related metals, form ferricyanides having similar properties; accordingly they give rise to structurally similar deposits, which under similar external

conditions exhibit similar behavior. It should further be noted that this behavior is itself a factor in the determination of the type of structure formed. The structure of the system and its physical activity thus mutually influence each other, and the eventual outcome in permanent structure deposited is the result of this continual interplay of chemical and physical factors.

The whole sequence of events in the formation of a single striated precipitation-vesicle of cadmium ferricyanide may be briefly sketched as follows (for further details see below, page 260). The original vesicle or tubule with which growth starts is formed at an anodal region on the surface of the metal; here cadmium ions enter solution and interact with ferricyanide anions to form cadmium ferricyanide, which is deposited as a thin easily ruptured semipermeable precipitation-membrane. The interior of this original precipitation-vesicle contains a soluble salt of the metal (*e. g.*, $CdCl_2$, formed from soluble chloride in the solution), and more precipitate is formed wherever the wall of the vesicle ruptures and the solution flows out from the interior to meet the potassium ferricyanide outside. The direction and rate of this outflow depend upon various physical factors (form and size of vesicle, character of local circuit, external conditions, etc.), which consequently determine where and at what rate new precipitation-structure is formed. The physical properties of the newly deposited precipitation-membrane, which by degrees builds up the wall of the growing vesicle, depend upon the specific chemical nature of the precipitate (since to a definite chemical composition correspond definite physical properties); these determine the properties of the wall of the vesicle, *e. g.*, its elasticity, coherence and resistance to rupture, and hence the rate of the rhythmical rupture; this again determines the rhythm in the outflow of solution, and consequently the rhythm of precipitation; and this latter rhythm determines the arrangement and spacing of the striations, *i. e.*, the characteristic morphological features of the completed structure.

This whole sequence may serve as a simplified model or epitome of what takes place in any organic formative process. In both the living system and its inorganic model the energy for the work of growth is derived from the energy freed in chemical

reactions. Certain insoluble and physically coherent products of these reactions form structure, which as soon as formed influences in a definite manner the chemical and physical processes by which further structure is formed. In its special character this structure varies with the chemical nature of the structural material; as is general with matter in the solid form, this material is deposited from solution in a crystalline or quasi-crystalline state of aggregation, which is structurally specific in the same sense as the molecular structure is specific. Thus the microscopic and ultimately the macroscopic form-characters of the growing system are determined by the special molecular structure of the formative material.

Organic growth, as Child has so clearly pointed out, depends upon the progressive accumulation of the solid metabolic products which are stable under the conditions. Since the chief of these structure-forming products, the proteins, have the most minute and detailed specificity known among chemical compounds, it is not surprising that the living systems built up from these compounds should be correspondingly individualized and specific in their structure and activities. The formation of precipitation-structures from metals may be regarded as a simplified model-process which makes it easier to understand why organisms whose proteins are similar in chemical configuration (as shown *e. g.* by precipitin tests) are also structurally and physiologically similar—*i. e.*, are closely related in the natural system. Chemical similarity and structural similarity go hand in hand, and structural similarity involves similarity in physiological activity and external behavior.

We have seen that the formation and activity of these precipitation-growths are the result of processes of electrolysis in the local electrical circuits arising between different areas of the metallic surface. The currents of these circuits control the structure-forming process; and in conformity with this condition we find experimentally that the formation of filaments can be promoted, modified, or suppressed at will by electrical means.[1] In this respect also an analogy to organic processes is to be seen. The electrical sensitivity of living matter is one of its most

[1] R. S. Lillie, *loc. cit.*, pp. 148–157.

characteristic peculiarities; and since the energy of any vital activity resulting from electrical stimulation—*e. g.*, muscular movement—is derived from chemical change in the tissue, it is clear that this sensitivity implies an influence of the electric current upon the chemical or metabolic processes by which energy is freed. These processes are mainly oxidations, and the energy thus freed may be applied in chemical and structural synthesis, as well as in motor or other activity. The facts of electrical stimulation, inhibition, and directive control (*e. g.*, in galvanotropism) show that the chemical reactions in protoplasm can be initiated, altered in rate, or suppressed by the electric current; and there is much evidence that the electric currents produced by living cells in their own activity exert normally a controlling influence of this kind. In the two preceding papers of this series I have briefly reviewed the known facts indicating that the organic growth-processes both give rise to and are influenced by electric currents.[1] The universal susceptibility of organisms to electric stimulation is in itself indirect evidence that normal growth is subject to this form of control, since functional activity, which is readily initiated by the current, has a profound influence on growth-processes. Bioelectric currents appear always to accompany normal functional activity, and many of the most fundamental cell-processes, such as the transmission of states of excitation in cells and nerve-fibres, appear to be directly determined by the currents of local bioelectric circuits.[2] A dependence of functional activity upon bioelectric processes implies a dependence of growth upon such processes, although further investigation is needed before the precise nature of this interconnection can be defined.

The recent interesting work of Child and Miss Hyman[3] has given further direct evidence of such an interconnection; they find that the most actively growing zones in various animals, especially annelids and planarians, are electrically *negative* to

[1] *Loc. cit.*, p. 181; BIOL. BULL., 1918, Vol. 34, p. 85.

[2] *Cf.* my recent series of papers on the general physiology of protoplasmic transmission: *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414; 1915, Vol. 37, p. 348; 1916, Vol. 41, p. 126; *Science*, 1918, N. S., Vol. 48, p. 51; *Scientific Monthly*, 1919, Vol. 8, p. 456.

[3] *Cf.* L. H. Hyman, *Science*, 1918, N.S., Vol. 48, p. 518.

other regions. The evidence now available indicates that this condition is a general one, and that in growing tissues and organisms (both animals and plants) the regions of preponderant growth are typically electronegative (in the physiological sense) to less actively growing regions, *i. e.*, at the actively growing regions the positive stream of the bioelectric circuit enters the living cells from the surroundings. It is well known that in irritable tissues the physiologically active or stimulated regions are negative to resting regions; growth appears to agree with other forms of functional activity in the character of its bioelectric manifestation. The fact that functional activity is in general a condition favorable to growth is probably to be connected with this fundamental similarity, and suggests that the bioelectric currents associated with activity have themselves a growth-furthering influence upon the tissue. Now organic growth implies the synthesis of specific compounds, especially of proteins; apparently therefore this synthesis is favored where the positive stream enters the protoplasmic surface from the outside. In the typical irritable tissue like muscle or nerve the bioelectric current leaves the irritable element at the resting regions and enters it at the active region; but activity at the latter region is usually temporary, unless stimulation is continued, and it is probable that the normal automatic return to the resting state is directly determined by the re-entrance of the current into the irritable element at the active area.[1] We know that a positive electrode from an external source of current has an inhibiting effect when applied to an active area of tissue, and the same effect must result at the entrance-region of the current generated by the tissue in its own activity.

All of these facts receive a consistent unitary interpretation on the hypothesis that the entrance of the electric current (positive stream) into the protoplasm induces or promotes certain oxidation-processes, primarily in the surface-film, and that these oxidations form a necessary condition of the synthetic processes by which the specific structural material of protoplasm is built up. In the actively growing cell or organism this synthesis

[1] The automatic repassivation of iron in strong HNO_3 offers a close physico-chemical analogy; *cf.*, my paper in *Science*, *loc. cit.*, p. 55.

results in a permanent increase in the quantity of living substance. In the stimulated irritable element (*e. g.*, muscle-cell) growth is not always evident; but there is evidence that the permeability of the surface-film is increased during stimulation (this structure being apparently broken down or otherwise altered in the stimulation-process), and it seems probable that the normal semipermeable condition is restored by a synthetic process of the kind indicated, and that in this manner the cell regains the resting state. According to this view the altered surface-film is restored to its original or resting state after stimulation by a process involving both chemical and structural synthesis, which takes place under the influence of the local biolectric circuit. Presumably repair of the cell-surface after local injury is accomplished in an essentially similar manner. The need of oxygen for the processes of growth, regeneration, or recovery from poisoning, narcosis or over-stimulation (fatigue) may be understood on this general hypothesis; in all such cases oxidation-processes occurring under the control of local bioelectric circuits are necessary for the syntheses involved in the process of construction or restitution.[1]

An instructive physico-chemical analogy is furnished by the local electrochemical reactions concerned in the transmission of the active state over the surface of passive metals, *e. g.*, iron,—a process apparently similar in many essential features to protoplasmic transmission.[2] Here the propagation of the local activation from region to region is directly due to local circuits formed at the boundary between the active and passive areas of the metallic surface; in an iron wire immersed in strong nitric acid the protective or passivating surface-film of oxidation-product is formed at one region of the surface (the anodal) as the activation-wave passes, and broken down by reduction at an adjoining region (cathodal). In such a wire any local inter-

[1] Loeb's observations on the breakdown of the cell-walls in the blastomeres of the *Ctenolabrus* egg in the absence of oxygen and their re-formation when oxygen is readmitted afford a striking instance of the dependence of structure-formation upon oxidation-processes. *Cf. Archiv. f.d. ges. Physiol.*, 1895, Vol. 62, p. 249; also in "Studies in General Physiology," University of Chicago Press, 1905, Vol. 1, p. 370.

[2] *Cf.* my discussion of this resemblance in *Science, loc. cit.*

ruption of the surface-film is thus at once automatically repaired, since that region, by the exposure of the underlying metal, is rendered anodal, and hence becomes the site of formation and deposition of the passivating surface-film. Similarly in the production of the local bioelectric circuit, *e. g.*, in a stimulated nerve, there is apparently a physical and chemical alteration of the protoplasmic surface-film, involving increased permeability, at one region, that of excitation; this altered region becomes electrically negative, so that the positive stream of the resulting bioelectric current there re-enters the protoplasmic surface; and apparently it there effects an oxidative synthesis which restores the resting condition. According to this conception, constructive or "anabolic" processes predominate at the regions where the current *enters* the protoplasm from outside; while at the adjoining inactive regions where the current *leaves*, and where a new state of excitation is automatically aroused, it is to be assumed that processes of the reverse kind take place.[1]

It is interesting to note that many years ago, before the development of modern physical chemistry, Hering reached very similar conceptions of the relation of the electric current to stimulation and to vital activity in general.[2] He rejects Du Bois-Reymond's purely physical conception of the origin and significance of the bioelectric currents, and recognizes that they are essentially indexes of chemical changes in the living matter. Similarly, external electric currents affect living matter primarily through their *chemical* or metabolic influence; where the current enters the protoplasm *assimilatory* (anabolic) processes predominate, and where it leaves, *dissimilatory* (catabolic); the stimulating and electrotonic influences of the current are referred to these characteristic influences on metabolism. These concep-

[1] Since such constructive processes are the condition of growth, we should expect, according to this hypothesis, that the constant electric current in influencing growth should *promote* the latter where the positive stream enters the tissue (*i. e.*, at the anode of the pair of applied electrodes) and *inhibit* where it leaves (at cathode). According to the recent observations of Bose (Proc. Roy. Soc., B, Vol. 9, p. 364) this is the case in plant growth. "In the polar action of electric current on growth, the anode is found to enhance and the cathode to depress the normal rate" (p. 399).

[2] Hering, "Theory of the Functions in Living Matter," *Brain*, 1897, Vol. 20, p. 232 (translation of article in *Lotos*, IX., Prag, 1888).

tions are surprisingly in accord with our modern physico-chemical point of view. The physiological polarity in the action of the current implies a polar difference in the chemical effects which it produces in living matter. The closest physico-chemical analogy is electrolysis; and it is to be inferred that processes of the same essential nature as electrolysis underlie the chemical effects of the electric current in organisms. This possibility was partly recognized by the older electrophysiologists,[1] but in the absence of any clear conceptions of the fundamental physico-chemical constitution of living matter they could do no more than make this suggestion and trust to later investigation and criticism for confirmation or disproof.

In the precipitation-structures described in this paper the new structural material is also formed at the anodal regions of the metal; and the inorganic growth-process at any region can be accelerated by making that region more strongly anodal, or inhibited by making it cathodal. The process of construction is thus susceptible to electrical influence, and this influence exhibits polar character—a necessary consequence of its dependence on electrolysis. Obviously the resemblance of the inorganic model to the living system relates only to the general nature of the conditions which control the chemical reactions underlying the formation and deposition of structural material. The detailed character of the formative processes in living matter can be made clear only by further investigation, but recognition of the general physico-chemical class to which the processes belong is essential as a guide in this investigation.

The senior author is responsible for Part I. of the present paper. The observations and photographs contained in Part II. were all made by Mr. Johnston, whose own account of his work now follows.

Part II. Experimental.

I. *Introduction.*

It is the purpose of this paper to describe in some detail various types of precipitation-structures produced by means of electrolytic local action in metals, according to a method de-

[1] *Cf.* E. du Bois-Reymond, "Untersuchungen über thierische Electricität," Vol. 2, p. 387.

scribed in a previous paper by the senior author.[1] In the present paper description of methods will be confined to certain modifications which have been found to be more suitable to the production of a certain definite type of structure. Certain conditions determining the type of structure produced have been studied in fuller detail. Expecially interesting is the fact that the process of formation is often periodic and accompanied by rhythmic motion of the structures formed. Regularly segmented or striated formations are thus often produced. Many interesting biological comparisons are therefore suggested.[2]

II. *Methods.*

If a piece of fine iron wire, wound about one end with a fine copper wire, be dropped into a 2 per cent. egg-albumin solution containing 4 per cent. K_3FeCy_6 and 4 per cent. or more of NaCl, the entire surface of the iron wire rapidly becomes covered with fine filamentous growths (Fig. 1). They are characteristically regular in form; the majority are straight or slightly curved and cease growth at a length of 200 microns or less. Very few, if any, of the larger striated or branching structures are formed under these conditions. A repetition of this experiment using a less concentrated solution of the salts (2 per cent. egg albumin containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl) gives a different result. The rate of growth is decreased, the form of the filaments is more irregular and many larger structures are formed (Fig. 2). In the stronger solution growth usually ceases in about five minutes, while in the weaker solution it may continue for several hours.

One method of controlling the rate of growth is to start the reaction with a quantity of the solution just sufficient to cover the surface of the metal and then to add from time to time with a pipette a few drops of the fresh solution. All of the available K_3FeCy_6 is soon transformed and the reaction ceases until renewed by adding more solution.

It is sometimes an advantage for the study of single filaments or other structures to limit the number formed from the metal.

[1] R. S. Lillie, BIOL. BULL., 1917, Vol. 33, p. 135.

[2] *Cf.* Lillie, *loc. cit.*, pp. 157–172.

This may be done by coating the metal with paraffin, and then, before placing into the solution, removing several very small areas. Only one or two structures may be formed from such exposed surfaces.

Experiments have been performed with the following metals: Fe, Zn, Cd, Co, Ni, Cu, Pb, Sn, Cr and Al. For each metal which forms a precipitate with K_3FeCy_6 there is a definite and characteristic type of precipitation structure.

The presence or absence of a protective colloid has a marked influence on the kind of structure formed. Definite tubular structures or filaments are formed from Zn, Cd and Co only in the presence of a protective colloid; in its absence most of the precipitate is deposited in an "amorphous" state. Copper differs from these metals in forming filaments readily in the absence of the protective colloid.[1] It forms its characteristic structures in a 4 per cent. solution of K_3FeCy_6 in distilled water containing 4 per cent. NaCl.

III. *Character of Structures Produced with Different Metals.*

1. *Iron.*—Iron gives rise to a greater variety of well defined precipitation-structures than any of the other metals used. The structure most frequently formed is a hollow filament circular in cross-section and somewhat tapering to the end. Such a filament has a typically straight or slightly curved form if the latter is not influenced by some modifying external condition. The smaller filaments are very easily influenced and are therefore less regular in the direction of growth than the larger filaments. Figs. 3, 4 and 5 show some of the typical iron ferricyanide structures. All were photographed from the same preparation at intervals of 10 minutes, 1 hour, and 24 hours, respectively, after placing in the salt solution (2 per cent. egg albumin solution containing 2 per cent. K_3FeCy_6 and 0.5 NaCl).

In the earlier stages of development these structures are thin walled, translucent, and comparatively smooth. Later the walls become thicker and more opaque. The filaments shown in Fig. 3 are all under the surface of the solution and the presence of a distinct double contour proves beyond doubt that even the

[1] Lillie, *loc. cit.*, p. 141.

very smallest of these structures are tubular. The walls of these filaments are precipitation-membranes; in the interior of the filament is a dissolved salt of the metal, which upon coming in contact with the ferricyanide solution outside forms the precipitate upon which the growth of the structure depends. A detailed description of this process as well as a very probable explanation of the cause of the outflow of the solution has been given by R. Lillie.[1]

The phenomenon of branching, although infrequent in iron filaments, occurs under certain conditions (Fig. 6). No branching has been observed except with the larger filaments and as a rule only with those which run along the surface of the solution. Some few cases, however, have been observed where the entire structure was submerged during its formation.

Slowness of growth is an essential condition for branching. Large filaments like those shown in Fig. 6 grow at the rate of 30 to 60 microns per minute, while the small filaments shown in the same photograph grow at the rate of 120 to 300 microns per minute. It required about 2 hours for the large branching structure shown in Fig. 6 to form. It is approximately 3 mm. long. The branches are always formed within a short distance of the growing end of the filament. It is probable that they are given off at thin or weak places in the walls where rupture and outflow of solution occur.

In general it has been found that the slower the rate of growth —that is, the nearer it approaches that of organic growth—the more resemblance there is to an organic structure. There is no doubt that the rate of the local chemical reaction, *i. e.*, the rate of formation of the precipitate, is an essential factor in determining the final form of the structure. In every case where there was a decided change in the rate of growth, there was a modification in form. The subject of the relation of rates of growth to form has been studied extensively in living organisms. D'Arcy Thompson,[2] in his book "Growth and Form," maintains that the final form which an organism assumes is entirely dependent on rates of growth. He says, "the phenomenon of rate

[1] Lillie, *loc. cit.*, pp. 144–146.

[2] D'Arcy Thompson, "Growth and Form," University Press, Cambridge, 1917.

of growth deserves to be studied as a necessary preliminary to the theoretical study of form, and, mathematically speaking, organic form itself appears to us as a function of time."[1] The rate of formation of all these structures, which depends on the rate of local electrolysis, is always most rapid at first and slows down as the reaction continues. Both internal and external conditions enter to cause the retardation. The frictional resistance to the outflow of the solution from the tubular filament and the electrical resistance of the circuit are proportional to the length of the filament. As these increase the rate at which the precipitate is formed decreases; the rate of growth is consequently lowered. There is an analogy here to the formation of living organic structures, where growth is always more vigorous at first and slows down as development continues. Child says, in his chapter on rate of metabolism, "everywhere the rate of growth is high in the young organism or in the young cells and tissues of the organism, and decreases as development proceeds and the rate of metabolism falls. With adequate nutrition and external conditions which permit growth the rate of growth appears to be in a general way dependent on the rate of metabolism";[2] and he points out that the accumulation of structure formed in metabolism may itself impede the metabolic process by which more structure is formed. The case of precipitation-structures offers an interesting analogy.

The influence of external conditions on rate and character of growth will be discussed below.

Branching can be produced in large filaments by perforating their walls with a finely pointed needle or glass rod. If this is done while the filament is growing, the branches resemble the main filament in almost every particular (Fig. 7). As many as seven branches were produced in this way, each new branch being formed from the preceding one, and sometimes three were growing at the same time. Puncturing the membrane after a filament has ceased to grow forms small side branches which themselves almost immediately cease to grow (Fig. 8).

Cross-striation is frequent in Fe filaments as well as in filaments

[1] Thompson, *loc. cit.*, p. 51.

[2] C. M. Child, "Senescence and Rejuvenescence," Chapter XI., the Rate of Growth, p. 276.

formed from other metals. This phenomenon occurs very regularly in the larger filaments that run along the surface of the solution, and occasionally in the smaller ones. The striations in the large filaments are usually about 25 microns apart (Fig. 9). No observation has been made on the precise mode of formation of striation in iron ferricyanide structures. Usually striation indicates periodic formation; and in the case of the similar striations in Cd or Zn precipitation-structures a direct connection with periodic precipitation can be observed. Probably the conditions determining the striation in iron filaments are essentially similar, although differing in detail.

Mechanical obstacles placed in the path of growing filaments have no appreciable influence on the regularity of striation. Fine grains of sand were sprinkled into the salt solution and the filaments allowed to grow out among them. The effects are shown in Fig. 10. Although the filaments were broken up and caused to branch, in some cases entirely enclosing some of the sand particles, striation continued as before. Fig. 11 is a photograph of filaments in which the direction of growth was changed by placing a few drops of water from a pipette in the solution just beyond the growing end of the filaments. A thin precipitation-membrane was formed over the end of the filaments and growth ceased for a moment. Soon the solution inside forced its way through the thin membrane and new filaments were formed which grew back into the more concentrated solution. The enlarged regions of these new filaments represent osmotic effects. It will be observed that striations are formed, but that the distances between the striæ are more variable (25 to 50 microns).

2. *Zinc.*—When a small strip of zinc, in contact with a nobler metal (Cu), is placed in a 2 per cent. egg albumin solution containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl the entire surface becomes covered within a few minutes with a dense network of filaments (Fig. 12). These structures are tubular and show some cross-striation, but differ in almost every other respect from the iron filaments just described. In minute structure they are coarsely granular with a less sharply defined outline, the double contour not showing except in a few of the

filaments which run along the surface of the solution. The manner of formation of zinc filaments, described in detail below, presents one of the most interesting rhythmical phenomena seen among any of the precipitation-structures. The rhythmic motion at the end of the filament is no doubt the cause of the regularly striated surfaces. These effects can scarcely be seen in a microphotograph on account of the coarse, thick walls of the filaments. In Fig. 13 some of the larger zinc filaments are shown. Some of these are very regularly and evenly striated. The enlarged place in one of the filaments is an instance of a broadening and flattening effect produced when a filament comes to the surface of the solution. Filaments in all cases are circular in cross section while entirely submerged in the solution.

Branching is a common feature of these large slowly growing zinc filaments. The entire outline of these structures is very irregular (Fig. 14); apparently there is a tendency to give off branches all along the course of growth.

Fig. 15 shows a part of an extremely large zinc ferricyanide filament which gave off many lateral branches. It reached a length of about 2.5 centimeters. These very large filaments grow so slowly that the deposit of precipitate can scarcely be seen under the low power of the microscope. The example shown was photographed after 24 hours in the solution. Branching can also be produced in these large growing filaments by puncturing with a fine pointed needle.

3. *Cobalt.*—In minute structure the cobalt ferricyanide precipitation-filaments resemble those formed from zinc. They develop best in a 2 per cent. egg-albumin solution containing 4 per cent. K_3FeCy_6 and 1 per cent. NaCl, and when the cobalt is in contact with copper or some other nobler metal. They resemble zinc filaments in the coarseness of the precipitate, which imparts a granular appearance and irregular outline to the filaments. There is also much non-coherent precipitate scattered along the sides of the filaments. Fig. 16 shows the characteristic structures. The manner of formation of the filaments seems to be the same as for iron filaments,—an even outflow of solution and regular deposit of precipitate. When the Co-Cu combination is put into a weaker solution (2 per cent. egg albu-

men containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl), numerous hollow, vesicular, shell-like structures (Fig. 17) are formed in addition to the slender tortuous filaments. These precipitation-vesicles closely resemble those formed on cadmium. They are finely and evenly striated, the striæ being only about 5 microns apart. They grow so slowly that no rhythmic motion can be detected, but it is probable that their formation is periodic, as in the case of the cadmium precipitation-vesicles.

A highly characteristic feature of the cobalt filaments is their tortuous, tendril-like form. They curl and wind in all directions without any apparent cause. Nickel filaments show a similar behavior. The nature of the precipitate formed, no doubt, has much to do with this phenomenon. The long filaments show no striation and there is no tendency to form branches.

4. *Cadmium.*—The most characteristic structures formed from this metal are hollow shell-like growths (Fig. 18). These form best in a 2 per cent. egg albumen solution containing 2 per cent. K_3FeCy_6 *plus* 0.5 per cent. NaCl. The structures formed under these conditions are striated and exhibit a very regular rhythmic motion (see below, page 259). They are of various shapes and sizes; occasionally they resemble a mussel-shell both in shape and character of striation. The striations vary in width in different cases from 20 to 30 microns. In Fig. 19 some of the same structures are shown after 24 hours in the solution. The largest of these are about 1 mm. in length. As is shown, their surface becomes entirely covered with fine short filaments giving them somewhat of the appearance of a burr. Very little growth takes place in the main structure after these fine filaments begin to form. As the reaction continues the enclosing membrane becomes thick and firm and more resistant to the forces which cause the rhythmic motion and periodic out-pushing of the structure. The small, short filaments then shoot out quickly at very regular intervals at different points over the surface. Some filaments are also formed directly from the surface of the metal. These are coarse and granular in appearance and show much non-coherent precipitate scattered along their course. If a strip of cadmium is put in contact with a nobler metal, or if a more concentrated solution of ferricyanide

is used, more filaments and fewer shell-like structures are formed, but in general cadmium shows little tendency to form filaments.

5. *Copper.*—Precipitation-structures are not formed so readily from copper as from Fe, Zn, and Cd and a few other metals. The solution-tension of copper is low, hence the metal does not readily enter into solution under the conditions of these experiments. A fine copper wire in contact with a nobler metal (Pt) or carbon, and immersed in a solution of 4 per cent. K_3FeCy_6 in distilled water containing not less than 4 per cent. NaCl, will in a short time form some filaments and numerous structures having the appearance of a burr (Fig. 20). The filaments that are formed are usually not large, are comparatively straight, and their walls are smooth and finely granular; very little non-coherent precipitate is formed. A few which run over the surface of the solution show a very perfect cross striation. None of these filaments show any tendency to branch. The burr-like structures are vesicular with short spicular filaments radiating out in all directions. They often exhibit a pulsating or rhythmic motion, one or more of the spicules shooting out rather quickly at each pulsation. On account of the darkness of color and thickness of the walls of these structures no striations have been detected, and few details can be shown in a photograph.

6. *Nickel.*—Strips of nickel in contact with copper or silver in a 2 per cent. egg albumen solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl soon form a great many winding tortuous filaments, closely resembling those formed from cobalt (Fig. 21). Some large, thick walled, irregular filaments also grow out very slowly from the surface of the metal, and large masses of amorphous precipitate are formed. Some of the filaments on the surface of the solution exhibit a few irregular cross striations. A few groups of small vesicular structures also form from the surface of nickel; these have a rhythmic motion during their formation and will be described later.

Experiments similar to the above were performed with Cr, Pb, Sn, Mn, Ag and Al, but no well defined precipitation-structures were formed except in the case of Cr and Pb. These two produced only a few very small filaments—so small in fact that they could not easily be studied under the high power of the mi-

croscope. Masses of amorphous precipitate were formed on Mn and Al, but Ag never showed any reaction, although left in the ferricyanide solution for hours at a time.

IV. *Conditions Modifying Form and Rate of Growths.*

1. *Electric Current.*—(*a*) The suggestion had been made that rate of growth of filaments could be accelerated or inhibited by electric influence.[1] Iron wires were made the electrodes in a ferricyanide solution by connecting to a battery through a rheocord, pole-changer and key. The strength of current required to affect the rate of growth is very small. By use of the rheocord the potential of the current could be altered at will. The general results were that both the rate of growth and the number of structures formed were accelerated or increased at the anode (Fig. 22) and retarded or decreased at the cathode (Fig. 23). By use of the pole-changer the current could be sent through the solution in the opposite direction, and the promoting and inhibiting effects would then show on the opposite wires. This experiment indicates clearly the electrical nature of the conditions controlling precipitation-formation from metals.

(*b*) A galvanic current from a storage battery was sent through a fine iron wire immersed in the solution. The result was both to increase the rate of growth and to change the shape of the structure. Upon making the current the rate of growth is increased and the growing filaments bulge to almost double their original diameter. By alternately making and breaking the current the filaments could be made to resemble a string of beads (Figs. 24 and 25). These effects are most probably due chiefly to the heating effects of the current.

2. *Sudden Changes of Concentration of the Solution.*—By removing the solution from around a filament-forming metal and then immediately replacing it with water the growing ends of filaments are caused to swell out in a manner shown in Fig. 26. After such an experiment growth usually ceases, and the walls of the filaments soon break down and fall apart in a peculiar manner as shown in Fig. 27. These are osmotic effects, and show that the walls of these structures are semipermeable.

[1] Lillie, *loc. cit.*, p. 157.

3. *Condition of Surface of the Metal.*—Oxide-covered areas on the surface of a metal form local cathodes in the ferricyanide solution; hence filaments are formed only where the pure metal is exposed. Fig. 28 shows a piece of rusty iron wire with the rust removed along one side; filaments were formed almost exclusively on the bright surface, which is anodic. Fig. 29 shows both ends of a fine iron wire (bent into a U-shape) 1.8 mm. apart, one end being rusty the other polished bright. Filaments were formed only on the bright end. It is interesting to note that nearly all the filaments formed on the bright end were directed toward the rusty end. Apparently the main direction of growth is determined by the direction of the current-lines between anodic and cathodic areas.

4. *External Conditions.*—All filaments are extremely senstive to outside influences. Slightly jarring or causing currents in the solution sometimes changes both the direction of growth and shape of filaments. Encountering small obstacles or other filaments has the same effect. Figs. 10 and 30 show the effect of filaments encountering small particles of sand.

5. *Modifications in Filaments at the Surface of the Solution.*—Some of these effects have already been mentioned. A widening and flattening effect is common with all filaments on reaching the surface of the solution. In some cases there is a display of different combinations of colors, and very beautiful figures of various shapes are formed. Filaments formed from nickel show no interesting features while entirely submerged in the solution, but when they run over the surface they become brightly colored, showing alternate light blue and brown cross-striations with a blue border along each side. Some interesting figures having the general shape of a bunch of leaves tinted with brown form at intervals along the filaments.

Another interesting surface-phenomenon is the formation of groups of small sac-like structures. This effect is very common with zinc filaments. As soon as a zinc filament in its tortuous course comes to the surface of the solution these small structures begin to form (Fig. 12). They push out around a common center, one forming every one or two seconds, at a rate varying somewhat in different cases. At the central point there is a

regular rhythmic motion which lasts for several minutes. A thin and almost transparent membrane swells up rather quickly and then a small saccule shoots out at the side; at this instant the membrane falls back, seemingly the pressure against it being relieved. This process continues for several minutes in each case and finally slows down and stops. The thin membrane never seems to rupture except at its edge where the little sac-like structures are formed. In some cases small filaments form also and radiate out around the membrane.

After several hours many such groups have formed and the whole presents a striking appearance. This phenomenon is not frequent with iron filaments, although some cases have been observed (Fig. 6). In these the vesicles are usually smaller, they form more quickly and a larger proportion of fine filaments are formed. Such structures are frequently formed from cobalt filaments and in this case are always much larger than those formed from iron or zinc (Figs. 16, 17). They vary from 200 to 300 microns in diameter, the enclosing membranes are thicker, and the whole structure is somewhat oblong in shape. The exact manner of formation has not been observed; no rhythmic motion has been seen.

6. *Influence of Mechanical Contact.*—Contact with solid objects has a marked influence on both the direction and rate of growth. In some cases filaments have shown tendril-like tendencies. Cases were observed in which Fe-ferricyanide filaments have wound spirally around a fine iron wire as often as nine times. Filaments of all the metals used show some reaction to contact with other bodies. Fig. 9 shows three filaments following along side by side for some distance.

7. The retarding and accelerating effects on the growth of precipitation structures observed in combinations of metals of different solution-tension have already been studied and described in full by R. Lillie.[1]

V. *Rhythmical Phenomena in Growing Filaments and Other Structures.*—Rhythmic motion is not evident in the formation of filaments in all cases, as, *e. g.*, in the filaments formed from iron. These seem to grow by an even and regular deposit of

[1] Lillie, *loc. cit.*, pp. 144–146.

precipitate, although, under certain conditions the surface filaments show a regular cross striation, a feature probably indicating periodic precipitation.

Under some conditions not yet clearly defined small burr-like structures are formed on the surface of an iron wire. Their formation seems to depend more on the condition of the surface of the metal than on the concentration of the solution. Some of these structures are shown in Fig. 2. They are always very small, and in the presence of a large number of filaments may easily be overlooked. During their formation they show a regular rhythmic motion causing the fine filaments on their surface to wave to and fro with a cilium-like movement. In some cases the whole structure pulsates, but in others only the filaments show any motion. This movement is comparatively slow, the rhythm being at first only about once every second, and later slowing down to once every five to eight seconds; sometimes this rhythmical motion continues for ten minutes. In the formation of zinc ferricyanide filaments very interesting rhythmical phenomena are exhibited. A small strip of zinc, in contact with a nobler metal (Cu, Fe), when placed in a ferricyanide solution becomes entirely covered with a fungus-like coat of filaments in a few seconds. At the extremity of each filament there is visible a regular rhythmic motion or pulsation, very similar to that described above as accompanying the formation of sac-like structures from zinc filaments at the surface of the solution. An almost transparent membrane swells out from the end of the filament, remains an instant, and then falls back quickly; then again the membrane slowly pushes out and the process is repeated. There is no visible evidence that the membrane is ruptured at any stage of the motion; neither can any suspended particles of precipitate be seen to flow from the end of the filament. The filament slowly increases in length, and the rate of increase seems to be proportional to the rate of rhythmical motion. It seems probable that each time the pulsating membrane falls back, it has just been ruptured at its edges, some precipitate being then formed and deposited. The rate of the motion varies from about once per second in the larger filaments to five or six per second in the small ones. No

such movement is seen in the large filaments on the surface of the solution. During the formation of some of the larger filaments having this motion, small spicule-like filaments grow out radially from the edge of the pulsating membrane. One or more forms at each pulsation until, when the filament ceases to grow, the whole surface is covered with the small sharp filaments and presents a bristly appearance.

A similar rhythmic motion has been observed during the formation of groups of small structures on the surface of nickel. Here also the pulsating center is present, but small saccules, about 30 to 60 microns in diameter, are formed around the membrane instead of small filaments as in the case of zinc.

The burr-like structures formed on copper have the same rhythmic motion as the small structures which sometimes form on iron. They exhibit both the pulsating movement of the vesicle as a whole and the cilium-like motion of the separate filaments. The rate of movement is usually about three per second.

Cadmium ferricyanide precipitation-structures have a more regular rhythmic motion than in any other case so far observed. The structures are large enough to enable the exact manner of formation to be seen. Each structure originates at a minute anodic region over which a small precipitation membrane is formed, and the succeeding increase in the size of this membrane takes place periodically. Growth is not all in the same direction; hence, characteristic shell-like structures are formed instead of filaments (Fig. 18). The increase in size takes place through a periodical rupture and reformation of the enclosing membrane. At each rupture a slight outflow of solution occurs, but a new membrane is at once formed, closing the opening; in this way the structure is enlarged by the amount of new membrane formed during the pulsation. When this increase in surface takes place each time at a region whose position (relative to the metal) remains unchanged, the structure formed is regular and symmetrical in shape. Sometimes, however, the rupture is first at one region and an instant later at another; this tends to make the structure irregular and asymmetrical in shape. The surfaces of the structures thus formed are finely striated, the striæ

varying in width from 20 to 30 microns. The direction and arrangement of the striations depend on the manner in which the growth has taken place. On the symmetrical structures the striations are regular and equidistant. On the whole these precipitation-formations bear a very decided resemblance to group of small shells.

Rhythmical movements similar to those observed in these precipitation-structures are met with in various other inorganic processes. Periodicity in purely chemical reactions and in the formation of osmotic growths, as well as in the growth and development of organic structures, has long been known. One of the best examples of chemical periodicity is seen in the so-called Liesegang rings,[1] which are formed by a diffusion-process associated with regular periodic precipitations. Leduc,[2] in his book "Mechanism of Life," gives a full chapter to periodicity in chemical and other phenomena. He describes certain osmotic structures as having rhythmic motion and as growing periodically.[3] In Child's book, "Senescence and Rejuvenescence," considerable space is given to the subject of periodicity in organisms.[4] In this discussion he shows that the growth of an organic structure is not an even, uninterrupted development, but is a largely periodic phenomenon. Another interesting and curious phenomenon is the periodic catalysis of hydrogen peroxide by mercury, as described by Bredig.[5] The liberation of gas in the catalytic action takes place intermittently and may continue at regular intervals for an hour or more. An electrical current of action is also produced resembling that produced in the rhythmic contraction of the heart, and can be registered in the usual manner by a string galvanometer. All of these purely chemical and physical phenomena are significant in that they point the way to a better understanding of the phenomena of rhythm and periodicity in living beings.

[1] *Cf.* Liesegang, "Beiträge zu einer Kolloidchemie des Lebens," Dresden, 1909; Stansfield, "Retarded Diffusion and Rhythmical Precipitation," *Amer. Journ. Sci.*, 1917, Vol. 43, p. 1.

[2] Leduc *loc. cit.*, pp. 67–77.

[3] Leduc, *loc. cit.*, p. 156.

[4] Child, *loc. cit.*, pp. 187–193.

[5] *Cf.* Bredig and Wilke, "Erregung und Beeinflussung katalytischer Pulsationen durch elektrische Ströme," *Biochem. Zeitscher.*, 1908, Vol. 11, p. 67.

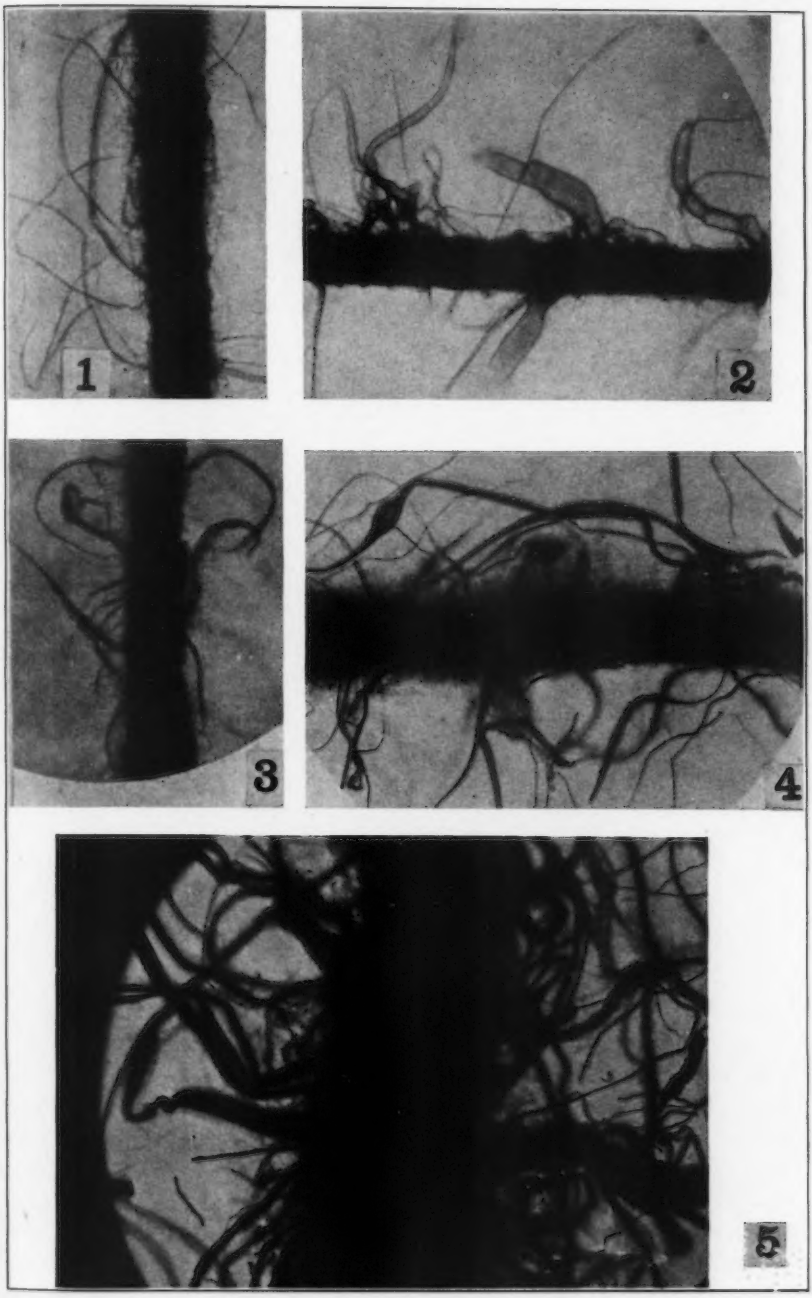
Description of Plate I.

Unless otherwise indicated the solution used for the production of the different structures was a 2 per cent. egg albumen solution containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl. In all these photographs the magnification is 32 diameters. In most cases the structures were allowed to develop while in position for taking the photograph, in order to avoid modifications produced by moving the preparation.

Fig. 1. Fe-ferricyanide filaments, 5 min., after placing in solution (2 per cent. egg-albumin, + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl).

Fig. 2. Fe-ferricyanide structures, about 10 min., after growth began.

Figs. 3, 4 and 5. Fe-ferricyanide filaments photographed after 10 min., 1 hr., and 24 hrs., respectively. From the same preparation.



RALPH S. LILLIE AND EARL N. JOHNSTON.

DESCRIPTION OF PLATE II.

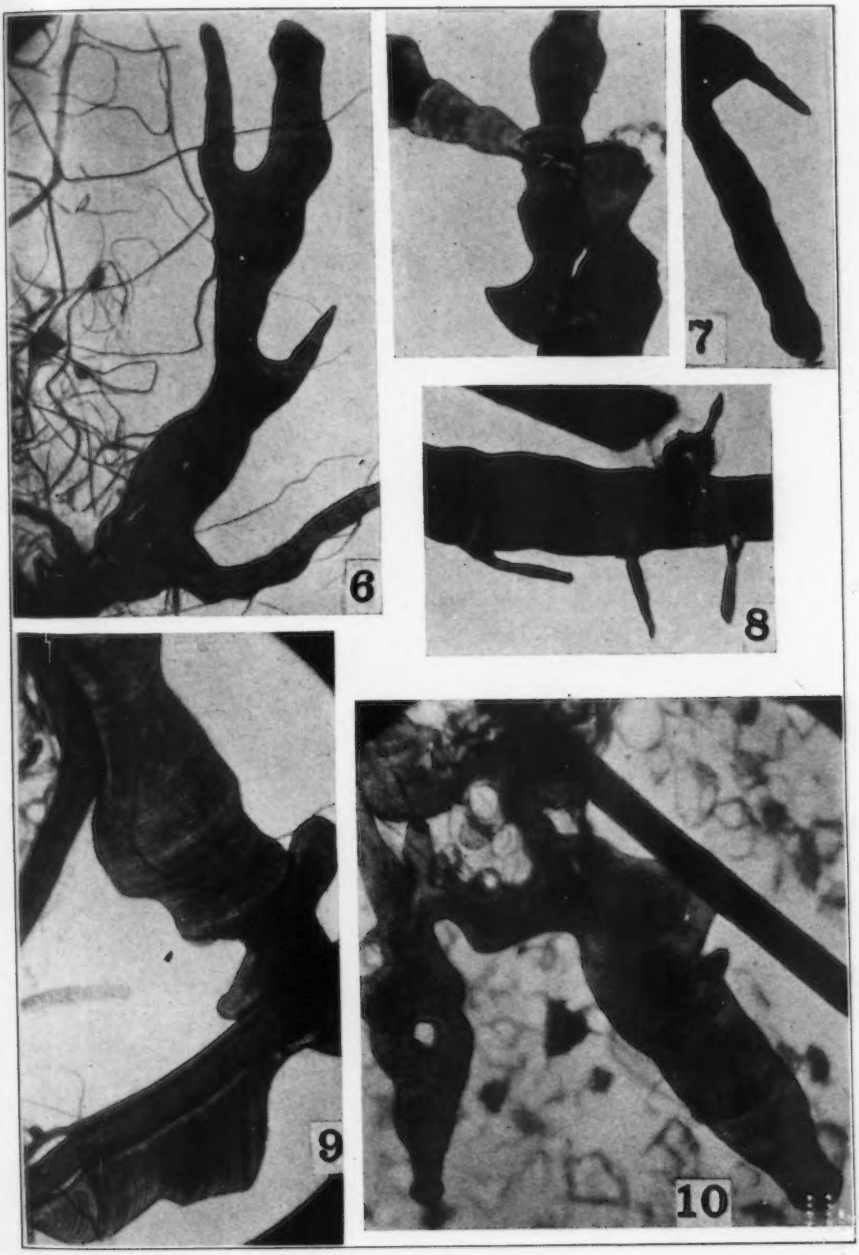
FIG. 6. Fe-ferricyanide structures, on the surface of the solution. Striæ about 50 microns apart.

FIG. 7. Branching produced in a growing Fe-ferricyanide filament by puncturing with a finely pointed needle. In the corner is a branch formed naturally.

FIG. 8. Branches produced by puncture in an Fe-ferricyanide filament after growth had ceased.

FIG. 9. Fe-ferricyanide filaments finely striated. Striæ about 25 microns apart. These filaments are on the surface of the solution.

FIG. 10. Large Fe-ferricyanide filaments, on the surface of the solution, broken up by fine particles of sand.



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DESCRIPTION OF PLATE III.

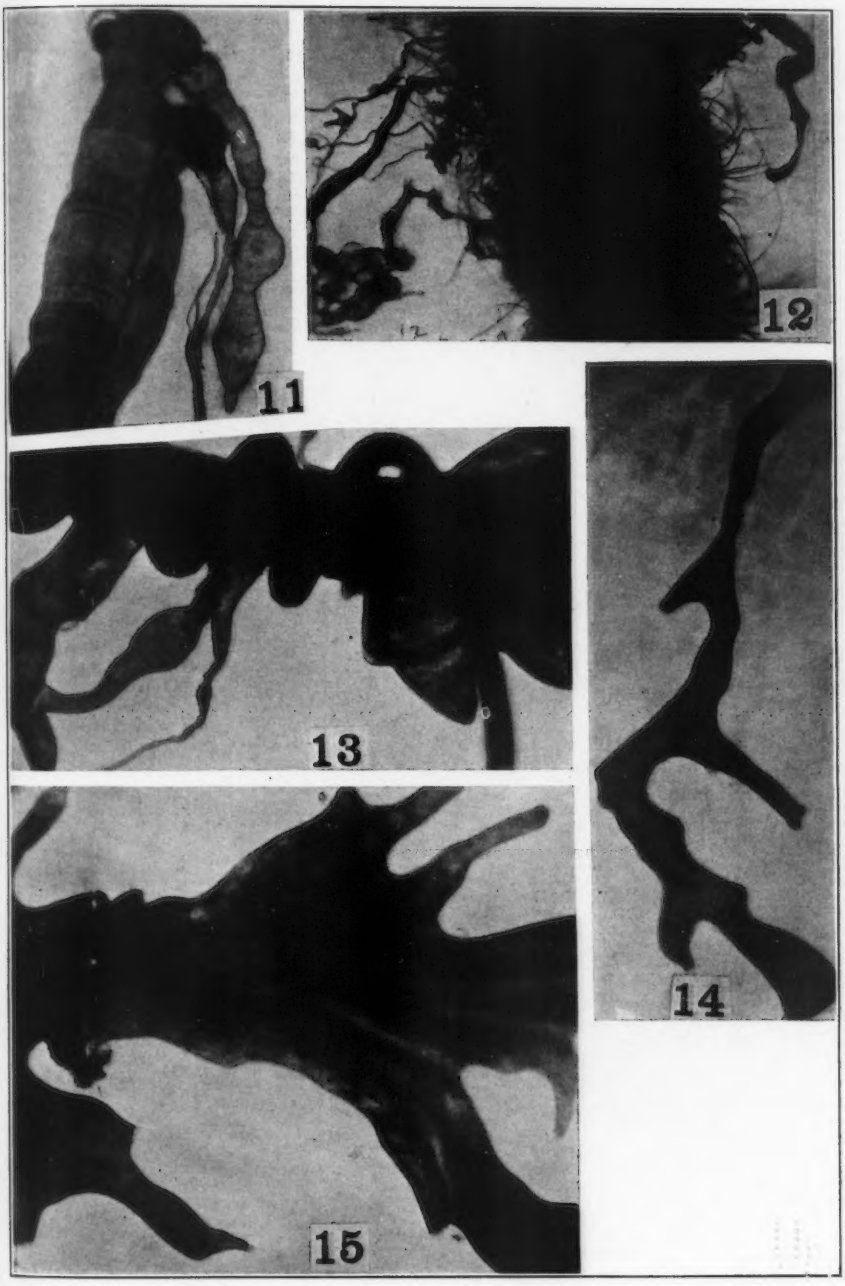
FIG. 11. Large Fe-ferricyanide filaments showing the effect of dropping water in the solution just beyond the growing end of the filaments. The striæ were 25 to 50 microns apart before the change of concentration.

FIG. 12. Zn-ferricyanide filaments, and saccules formed at the surface of the solution.

FIG. 13. Large striated Zn-ferricyanide structures. The copper wire is shown wound about the Zn-strip.

FIG. 14. Zn-ferricyanide filament showing the irregularity of growth at the surface of the solution and the general tendency to branch.

FIG. 15. Part of a large, branching Zn-ferricyanide filament on the surface of the solution. Photograph after 24 hrs., in the solution.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DESCRIPTION OF PLATE IV.

FIG. 16. Co-ferricyanide structures formed in 2 per cent. egg albumin + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl. A group of surface saccules shows at one side.

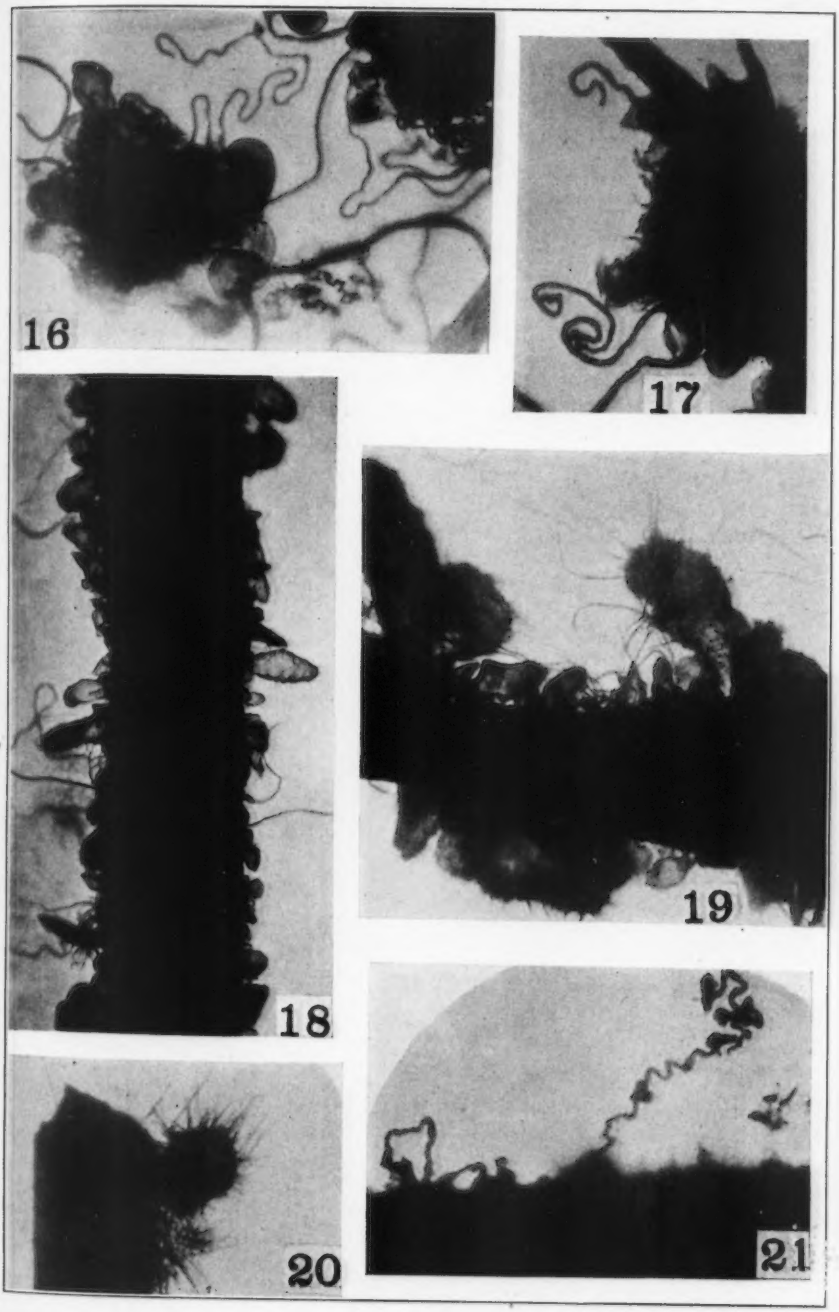
FIG. 17. Co-ferricyanide filaments and striated shell-like structures at the surface of the metal. The tortuous filaments are submerged in the solution.

FIG. 18. Cd-ferricyanide structures. The longest of these structures are about 320 microns. It required about 2 hrs., for these to form.

FIG. 19. Cd-ferricyanide structures photographed after 24 hours in the solution The largest are 1.1 mm. long.

FIG. 20. Cu-ferricyanide structures. Formed in a 4 per cent. solution of K_3FeCy_6 in distilled water + 4 per cent. NaCl.

FIG. 21. Ni-ferricyanide precipitation filaments. Formed in a 2 per cent. egg albumin solution + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl.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DESCRIPTION OF PLATE V.

FIG. 22. Fe-ferricyanide growths, showing the acceleration caused by making metal anodic while the structures are forming.

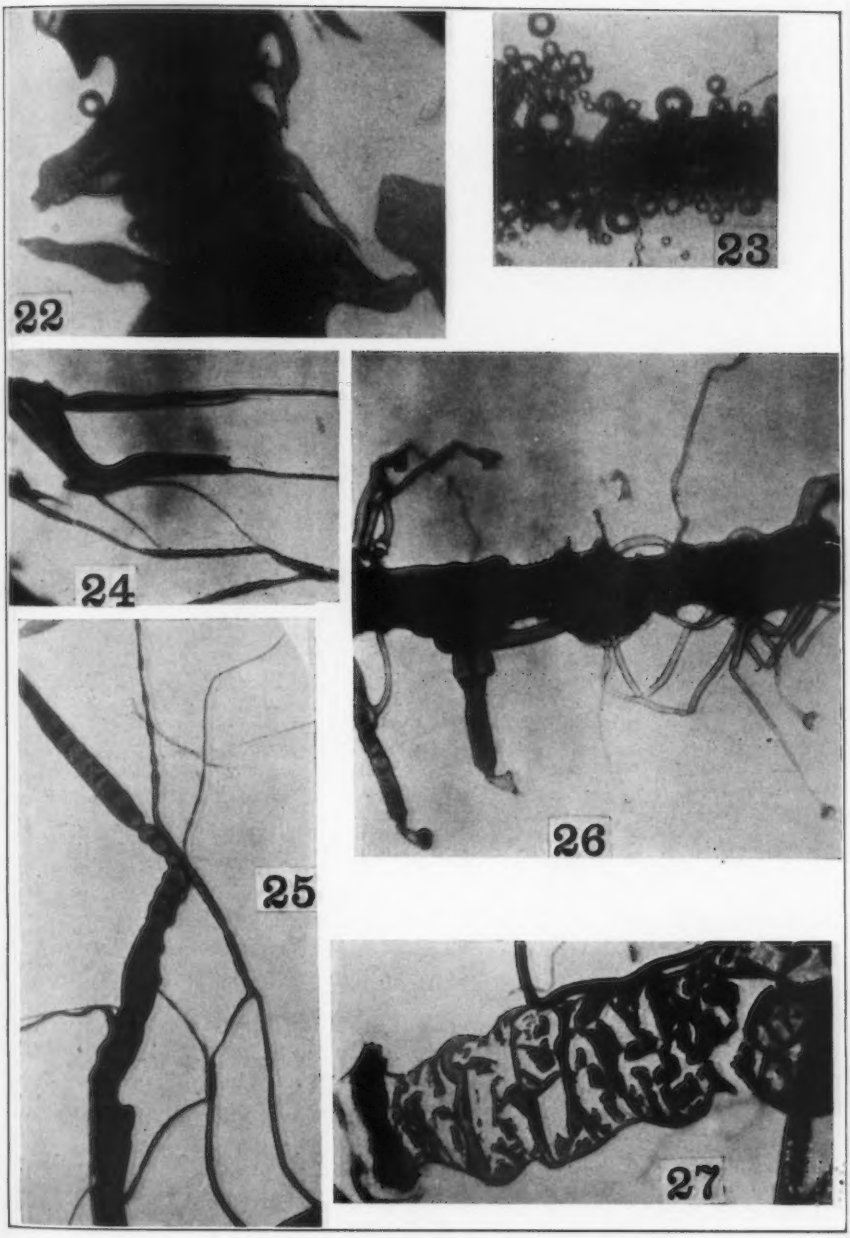
FIG. 23. An iron wire in a ferricyanide solution showing cathodic inhibition of precipitation formations. The round objects are hydrogen bubbles.

FIG. 24. Filaments showing modifications produced by passing a galvanic current directly through the metal while the filaments are forming. The beaded effect is produced by alternately making and breaking the current.

FIG. 25. Same as Fig. 24.

FIG. 26. Precipitation filaments from iron, showing enlargements at the ends of the filaments produced by a sudden decrease in the concentration of the solution.

FIG. 27. This shows the peculiar manner in which large Fe-ferricyanide filaments break down after a sudden lowering of the concentration of the surrounding solution.



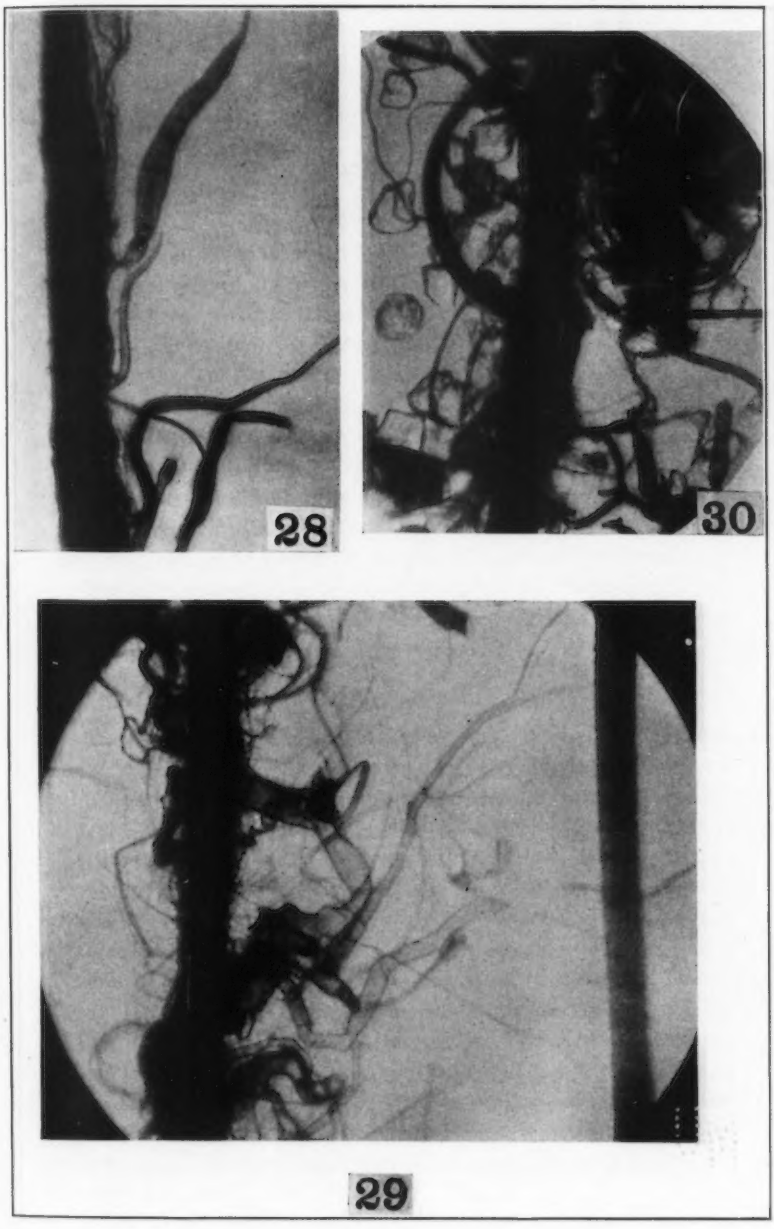
RALPH S. LILLIE AND EARL N. JOHNSTON.

Description of Plate VI.

Fig. 28. A rusty iron wire forming filaments only on the bright surface from which the rust had been removed. All rust-covered surfaces are cathodic.

Fig. 29. Both ends of a U-shaped iron wire in a ferricyanide solution. One end is rusted and forms no filaments, the other is polished bright, hence is anodic and forms many filaments.

Fig. 30. This shows the modification in direction of growth and shape of filaments caused by small obstacles (fine sand) in the solution.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TWO CILIATA OF GREAT SALT LAKE.[1]

DEAN A. PACK.

INTRODUCTION.

Great Salt Lake is not a body of water without its natural fauna and flora. Its water has not yet reached the point of saturation for sodium chloride. It is not the lake of no life, whose water is thought, by the public in general, to be purified by the abundance of salt in solution. It is a lake that contains probably less than fifty forms of life, some of which are visible to the naked eye. Upon careful microscopic examination of a generous amount of the Great Salt Lake water (23 per cent. salinity) many forms of life are observed. To date there have been seventeen different forms reported; as nine algæ, five bacteria, two protozoans, one crustacean and two fly larvæ.

The plan of work was to study the effect of dilution on Great Salt Lake forms. Out of a number of algæ and protozoans, two ciliata were chosen for experimentation.

MATERIAL.

The material was collected early in September, 1917, from near the shore of Great Salt Lake in the vicinity of the Salt Air Pavilion and permitted to stand in the laboratory until January, 1918. As no water was added to the material, it concentrated to a density of 1.170 at 60 degrees Fahrenheit, by the first of January, 1918, when the first dilutions were started. A good balance between the animal and plant life was established and the cultures retained their clearness through all the different densities from the point of saturation down to the density 1.015 (at date).[2] The decaying and milky cultures spoken of by other observers were not encountered. The *Aphanothece* and some other plants grew and reproduced, in all the different densities from 1.20 to 1.010, thus serving as animal food and giving the cultures their characteristic lake green color.

[1] Contribution from the Zoölogy Department of the University of Utah.

[2] August 1, 1918.

The number of living organisms appearing was so large in the first cultures, that contamination was suspected. Consequently collections for checking were made April 30, 1918, under all the precautions that one takes in the collection of water samples for bacteriological examination. The material was examined and found to contain the two ciliata described later in this article and some other forms in common with the September material.

Dilution was effected gradually and accurately, by a method of continuous diluting. The process consisted in adding enough distilled water to a culture to lower its density .0025 or one fourth of one per cent. every 24 hours. This method was adopted for several reasons; first, one does not destroy delicate forms that would perish when plunged into water of only one tenth the original density; second, one has before him a continuous picture of the changing life; and third, one deals with the offspring of only a few individuals.

The moist chamber made by the use of the hollow-ground slide was found to be an advantage in the study of these forms. It was made by placing a small quantity of culture, that showed an equal balance between animal and plant life, on a clean cover slip; inverting this over a deep hollow-ground slide, and sealing with vaseline. If small cultures are desired several may be made on one cover slip by means of the platinum loop. In this case a drop of culture is placed in the hollow-ground slide to prevent evaporation. A mechanical stage greatly facilitates the study.

Cultures with a predominance of animals or plants will show the effect of different gases upon the form and habits of both animals and plants. Not only can the effect of gases and liquids on living forms be studied to good advantage, but this preparation makes possible accurate study of responses to light, darkness, heat, etc. Some of these preparations were made fifty days ago and are at date in good condition.

General Discussion.

Diluting the culture medium produced certain definite changes in these two ciliata. These protozoans were found to vary with a change in the supply of food, oxygen, carbon dioxide, and light;

and also with respect to division, conjugation, and encystment. Dilution of the culture medium was accompanied by increased size, increased activity, shortening of the feeling cirri, more active physiological and reproductive processes, and more flexible and contractile bodies.

In changing the density of the culture from 1.110 to 1.040 the Uroleptus packii increased in length from .07 mm. to .11 mm. and the *Prorodon utahensis* increased in length from .06 mm. to .08 mm. The *Uroleptus packii* cysts increased in diameter from .025 mm. to .03 mm., and the *Prorodon utchensis* cysts increased in diameter from .023 mm. to .027 mm. Like changes were also noted among other protozoans.

The protozoans showed an increased activity as the medium became less dense. All estimates of speed were made by means of stopwatch, camera, lucida, and a dial made of concentric rings reading to .01 mm. and .05 mm. The rate given for each protozoan is the average of ten records for each of ten individuals. These rates are given in the following table.

Protozoan.	Date.	Density of Medium.	Distance.	Time.	Kind of Motion.
Uroleptus packii.....	May 20	1.2	100 microns.	10 min.	steady.
"	" 25	1.11	" "	7.5 sec.	"
"	" 25	"	" "	5.6 "	darting.
"	" 25	1.06	" "	3 "	steady.
"	" 29	1.04	" "	2 "	steady.
"	" "	1.06	" "	1–2 "	darting.
Prorodon Utahensis.....	Apr. 29	1.2	100 microns.	20 min.	steady.
"	May 25	1.075	" "	3.5 sec.	"
"	" 25	1.045	" "	3 "	"
"	" 25	1.039	" "	2 "	"

This increased activity was also very interestingly demonstrated by the rate of vibration of the feeling cirri on the *Uroleptus packii*. These ciliates, in the densities below 1.030, moved their feeling cirri hundreds of times faster than the same ciliates moved their feeling cirri when in the saturated densities. At a density of 1.22 one single stroke of the feeling cirrus occupied 15 seconds. One stroke of a feeling cirrus at the density 1.150 occupied 1 second. In the density 1.11 there were at least five strokes per second, while in the densities below 1.030 not even an estimate could be given. The increased activity, that came

with less dense media, was evident in other general movements.

The feeling cirri on the ciliates from the saturated medium were between 17 and 20 microns long, while the feeling cirri on equally large ciliates from a medium with density 1.030 were between 10 and 11 microns long. The two inside feeling cirri of the ciliate in saturated medium were thus reduced by dilution to nearer the length of the two outside ones and the ventral creeping cirri. That is, our ciliate has practically replaced the two specialized feeling cirri by the less specialized cirri.

The physiological and reproductive processes were accomplished in less time in the less dense solutions. With increased dilution; the protozoans ingested more food, the waste vacuole discharged more often, and the contractile vacuole hurried up in a like manner. Reproduction occurred more rapidly and more often as the foregoing processes were speeded up.

The bodies of both protozoans became markedly less rigid as dilution went on. In normal lake water the bodies of both forms were not known to contract any appreciable amount. *Prorodon utahensis* could bend its body through an angle of 30°, while the *Uroleptus packii* was never found in a bent position. At the density of 1.06 the *Prorodon utahensis* was seen to bend through an angle of 180°: or until the anterior part of the body folded on the posterior part. At the density 1.03 the *Uroleptus packii* was seen to bend through an angle of 150°. Both forms in the lower densities could shorten and lengthen the body at will. The *Prorodon utahensis* seemed to have lost all rigidity of body, assuming readily a spherical or a cylindrical form.

These protozoans responded definitely toward light. This fact was demonstrated, first with active and second with encysted forms. The active forms after an exposure to darkness for 12 hours, swam 66 microns in 10 seconds; while after an exposure to darkness for only 6 hours, they swam 100 microns in 10 seconds. The same forms after being exposed to darkness for 12 hours followed by exposure to light for 4 hours swam 300 microns in 10 seconds. These figures show: first, that exposure to darkness was accompanied by decreased activity; second, the longer the exposure to darkness the greater the inactivity; and third, that light was necessary for maximum activity. The

encysted forms for several hours before emerging exhibited periods of rest followed by periods of activity. This could be explained as fatigue. However it was found that they were more active during the day than during the night. It was also noted that individuals quieted down by exposure to darkness were excited to activity in a few minutes by exposure to a 40-watt light (distance 2 feet). These individuals were found to be more active as the intensity of light increased within the limits of photosynthesis. A suggested explanation of these results is found in the fact that the protozoans possess chlorophyll, probably in the form of a symbiotic alga, which gathers carbon dioxide and other wastes from the ciliate, recombines them into food, and liberates oxygen. The utilization of the synthesized food and free oxygen by the protozoan may account for its increased activity.

The geological history tells us that a few hundred thousand years ago Great Salt Lake was a body of fresh water. This suggests that our present salt water forms were in the past fresh water animals. The fact that these forms have withstood the the great change from fresh water to a solution of 23 per cent. salinity shows that they were capable of adaptation. Those forms that were unable to adapt themselves to the salt water have been weeded out and only plastic forms left. The results discussed in the foregoing part of this article show that these ciliata are plastic and capable of great adaptation. They adapted themselves to all densities from 1.22 down to 1.010 (to date), which is almost a complete reversal of a change covering thousands of years.

Description of Species.

Uroleptus packii Calkins[1] (see Fig. 1) is a pale green colored ciliate with dorsal and ventral surfaces, a tapering posterior end, and a blunt anterior end which bears two long and two shorter feeling cirri. This form is .07 mm. long (salinity 23 per cent.). The body is quite rigid; keeping the characteristic shape. The ectoplasm is easily distinguished and carries three marked rows of creeping cirri. These rows of cirri extend diagonally back-

[1] Prof. Gary N. Calkins has seen this form and, provisionally, named it *Uroleptus packii*.

ward from the right anterior end to the left posterior end over the ventral surface. The row fartherest to the left is interrupted one-third of the way back by the peristomial region. This region is bounded on the left by the peristomial membranellas,

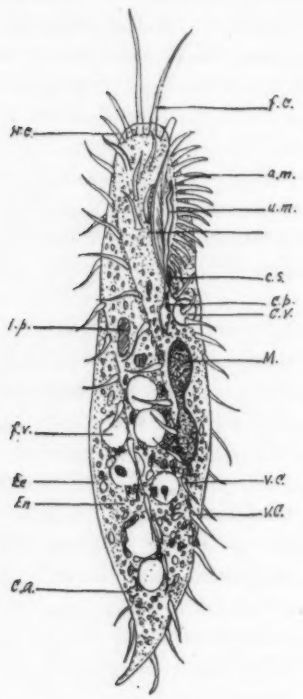

FIG. 1.

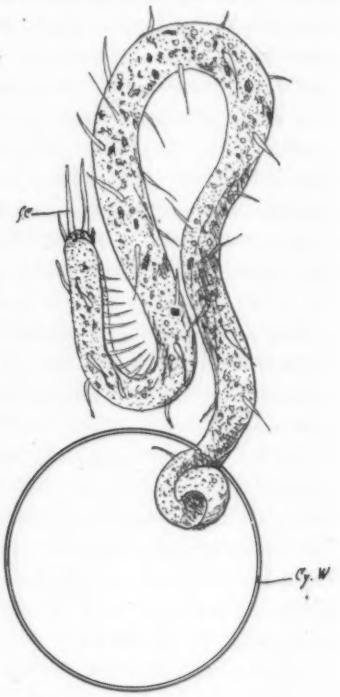

FIG. 2.

FIG. 1. *Uroleptus packii.* *fc*, feeling cirri; *frc*, frontal cirri; *am*, adoral membranellæ; *uml*, undulating membrane (tongue); *um*, undulating membrane; *cs*, cytostome; *cp*, cytopharynx; *cv*, contractile vacuole; *ip*, *Aphanothece* cell; *M*, macronucleus; *fv*, food vacuole; *Ec*, ectoplasm; *En*, endoplasm; *vc*, ventral cirri; *ca*, cytopyge.

FIG. 2. *Uroleptus packii.* *Fc*, feeling cirri; *cyw*, cyst wall.

which extends backward turning slightly to the right, passing through the mouth, and ending in the cytopharynx. The right side of this region is bounded by a large undulating membrane which extends backward and turns slightly to the left. A

second tongue-like undulating membrane extends out of the mouth and forward along the floor of the peristomial region. The cytopharynx is a short swollen tube that extends from the mouth backward with a left dorsal deflection. There may be as many as thirty food vacuoles in the body at one time. The remains of the food vacuoles pass out through the posterior dorsal anus. The contractile vacuole is situated in the fore part of the body. The nucleus is situated midway along the body in the left dorsal quarter. This form derives its color

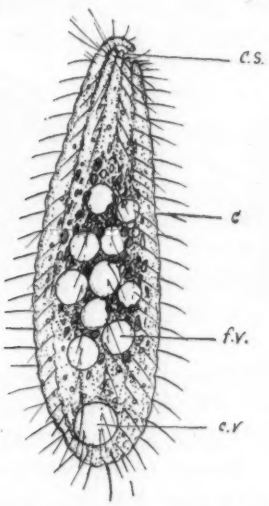

FIG. 3. *Prorodon utahensis*. *cs*, cytostome; *c*, cilium; *fv*, food vacuole; *cv*, contractile vacuole.

from chlorophyll, which is probably in the form of symbiotic alga.

This form takes food in the form of bacteria, small plants and animals which abound in the water. It is a very active form, having a creeping or swimming forward movement of 100 microns per 7 seconds and a darting backward movement of double this speed. This backward movement generally follows irritation at the anterior end. This form when kept in a saturated solution was found to be sensitive to a flash of light from the mirror of the microscope.

Reproduction is brought about by binary fission. Both transverse and longitudinal methods of fission were observed. Conjugation is followed by rapid division. At certain times, perhaps after a full meal, this protozoan encysts and may remain encysted for fifty days. It finally becomes active again and breaks through the cyst wall as a much elongated form (see Fig. 2). After swimming a short time in this form it divides transversely, and if the posterior individual is not sufficiently organized it rounds up into a cyst.

Prorodon utahensis[1] (Fig. 3) is cylindrical in shape, .05 mm. long, and bears an even coat of cilia. The cilia are distributed

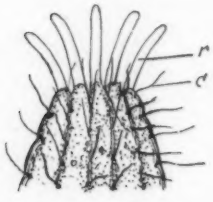

FIG. 4.

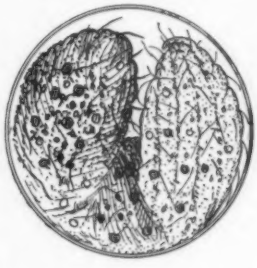

FIG. 5.

FIG. 4. *Prorodon utahensis*. Anterior end showing *r*, extended rods.
FIG. 5. *Prorodon utahensis*. Encysted.

between ridges that run parallel to the long axis of the body. These ridges project slightly beyond the opening of the mouth (anterior end) and form finger-like projections. One of which is long and well developed, being used to crowd food into the mouth. The œsophagus is short and fitted with short rods that may be extended in the shape of a star (see Fig. 4). The mouth and œsophagus are capable of being expanded to near the diameter of the body. Food vacuoles are many in number. The contractile vacuole is large and at the posterior end. The nucleus is small. The endoplasm probably contains symbiotic alga.

This form is an active feeder. It lives on bacteria, plant cells, amœbæ, small ciliata, mastigamœba, and other small protozoans found in the lake. An amœba, with a diameter fully equal to

[1] The writer has given the name *Prorodon utahensis* to this form, which is very probably a new species.

that of this ciliate, lost its identity two minutes after being swallowed. At one time an amœba was swallowed that occupied fully one-half of the protozoan's body. It may feed for several hours, then quiet down, contract into a short ellipsoid during which time it extends at intervals the œsophagus rods. After about one hour of this procedure it elongates and swims away, continuing again the process of feeding.

It is an active swimmer. Reproduction is brought about in a short period, by longitudinal fission. Cysts were observed and the contained individuals were found to be sensitive to light, a fact discussed in connection with symbiotic alga.

Conclusions.

1. Though the number of animal and plant species reported from Great Salt Lake is only seventeen, evidence goes to show that this number will at least be doubled.

2. The milky cultures are the result of an unbalanced animal and plant relationship.

3. Continuous dilution was an advantage in that it did not destroy delicate forms, and the offspring of only a few individuals were studied.

4. The two ciliates responded to dilution of the medium by increased size, increased activity, shortening of the feeling cirri, more active physiological and reproductive processes, and more flexible and contractile bodies.

5. These protozoans contain chlorophyll, probably in the form of a symbiotic alga.

6. The results of the foregoing experiments show that these ciliates are plastic and capable of adaptation.

7. It is believed from the foregoing that by slowing down the rate of dilution, some of these Great Salt Lake forms may be transformed into fresh water animals.

8. Most cordial thanks are due Professor Newton Miller, who followed the work with interest and gave many helpful criticisms.

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UTERINE, TUBAL AND OVARIAN LYSIS AND RESORPTION OF CONCEPTUSES.[1]

ARTHUR WILLIAM MEYER.

It has long been known that considerable intrauterine retrogression of a conceptus can occur in multiparous mammals. D'Outrepont, for example, on page of the 192 catalog of his collection, is said to represent the uterus of a rabbit, in which an apparently full term fetus is found near the distal end of one horn, although the approximate portions of both horns contained nothing but remnants of fetuses—one in each. According to Muller, '47, the enlargement in the right horn contained a "cyst filled with rabbit hair imbedded in a soft mass and that on the left side a similar convolute of hairs without a cyst." This, as far as I know, is the earliest reference to, even if not the earliest observation upon a fetal retrogression. But since rabbits are born naked, one is left to speculate upon the validity of this observation. However, that the idea holds is indicated also by some experimental work. Sokoloff's, '96, meager report, for example, seems to indicate that in dogs bilateral ovariotomy leads to the death of the embryo and to abortion of it and of the entire conceptus. Strahl and Henneberg, '02, also found that conceptuses in different stages of retrogression occur quite commonly among normally developed ones in the ferret, marmot and mole. They also found that the entire placenta and probably also the fetal membranes, as stated by Hubrecht, normally are retained for some time, even up to a month after parturition in the mole. Similarly, Frankel, '03, was able to cause the death and also the uterine absorption of conceptuses in rabbits up to the 20th day of pregnancy, through destruction of the corpora lutea. Frankel found that after 14 days, all that remained in the way of evidence of some pregnancies was an anemic ring which disappeared completely within three weeks. Henneberg,

[1] From the Department of Embryology of the Carnegie Institute and The Department of Anatomy of Stanford University.

'03, also found that intrauterine death and retrogression of the guinea pig fetus can be effected experimentally through various means, and according to Koebner, '10, not merely ova or young fetuses are absorbed, but even the bones of older ones disappear completely under experimental conditions in the rabbit. It would seem unlikely, however, that such could be the case in any but the very earliest stages in the development of the skeleton for a considerable degree of acidity would have to develop in order to make this possible. Since it is believed that the development of acidity not only is slow but also slight in embryonic tissues, Koebner's conclusion regarding the bones seems to be open to some doubt. Wiener, '05, also found that autolysis is very slight in tissues with an alkaline reaction and that a slight increase in acidity greatly accelerates it.

Fraenkel, '10, extended and confirmed his work done in '03 by a very large series of experiments touching various phases of the corpus luteum problem and among other things concluded that one corpus luteum can protect at least three ova sufficiently to insure continued development.

Although the ova found by Huber, '15, were very young, the significance of the facts would seem to be similar. Likewise Mall, '15, while writing "On the fate of the human embryo in tubal pregnancy," stated that "we have no data on the number of ova which disintegrate early, but the study of comparative embryology warrants the conclusion that many young ova degenerate and disintegrate. I am informed by Dr. Huber, who has studied with great care much material among rats, that some of the fertilized ova break down before implantation. The same seems to be true regarding the pig. We usually find more corpora lutea in the ovaries than embryos in the uterus, indicating that all of the ova do not produce normal embryos." Similar phenomena also were reported by Meyer, '17, in regard to young conceptuses in the guinea pig. Curtis, '15, also reported the absorption of ova in guinea pigs in consequence of the injection of extracts of the human placenta, but unfortunately did not give convincing evidence regarding his knowledge of the presence of pregnancy in the animals concerned, and did not make a microscopic examination. Since Curtis stated that

injections of defibrinated human blood had the same effect as the injection of extracts of the human placenta, and that the injection of extracts of guinea pig placenta and of guinea pig blood had no effect whatever, one can not help but feel decidedly skeptical regarding the trustworthiness of his experimental proof.

It is well to remember in this connection that phenomena which occur in other mammals, or conclusions drawn from these phenomena, must be used with caution when referred to man. The uteri of other mammals well may show a greater tolerance to the presence of dead fetal tissue and also greater resorptive power. Robinson, '04, stated that Vernhout showed that maternal tissues are not shed at the time of birth in the mole, and that some of the fetal tissues are retained to be absorbed later. Hill, it seems, found the same thing to be true in *Perameles* and *Dasyurus*. Jenkinson, '13, also stated, that in *Perameles*, the allantois and its blood vessels regularly are absorbed through the agency of maternal leucocytes by the parturient uterus and that fetal tissues are absorbed somewhat similarly in *Dasyurus*. However, since the young of some marsupials are in a very immature state during the first months of gestation and are then transferred to the marsupium as naked little fetuses said to be only about one inch long in the kangaroo, it is clear that absorption of the secundines at this stage of development would be something quite different from their resorption at the end of the gestation period in other mammals.

Although we have considerable evidence regarding retrogression and the partial or even the total intrauterine absorption of conceptuses in various mammals, I have not been able to find any conclusive evidence in the literature regarding the occurrence of this phenomenon in man. It is true that cases of Frankel, '03, Polano, '04, Rosenkranz, '03, and also cases reported by others are referred to as such examples, but a careful examination of the reports shows that those cases hardly can be regarded as falling under the head of intrauterine absorption of ovum, embryo or fetus. It is true that in the cases of Polano and Rosenkranz, the skeletal elements only were seen to have been discharged, but in the case of Polano the amniotic fluid never-

theless may have carried a great many fragments of embryonic tissue away with it beforehand. Furthermore, Polano does not claim his case as one of complete intrauterine absorption but merely as one of remarkable intrauterine maceration under aseptic conditions.

The history of the case of Rosenkranz, on the other hand, shows quite clearly that the fetus was destroyed by putrefactive changes. Rosenkranz himself emphasized this, but strangely enough ruled out entirely the occurrence of maceration. Rosenkranz further stated that the patient herself noticed the discharge of some bones with the "menstrual" flow. However, under the above conditions the recurrence of true menstruation is exceedingly unlikely unless the fetus was dead a considerable period before the rupture of the membranes occurred, and the placenta had been detached at least partly, for only under such circumstances could considerable regeneration of the mucosa occur and thus make the return to normal menstruation possible. It nevertheless is possible, however, that the time of abortion was co-incident with the date on which menstruation might have recurred. A small number of cases in the Mall Collection give a history which makes such a suggestion probable. Just why the expulsion of a dead, retained conceptus should occur at the time when menstruation would have recurred normally had pregnancy not supervened, is difficult to say, but since the inhibitory effect upon the menstrual cycle exercised directly or indirectly, by the living fetus is absent in cases of premature death and retention, it is possible that the abortion might occur at a time when the impulses of a return to the normal nonpregnant status of the maternal organism becomes more evident; that is, at the time of the recurrence of the normal menstrual cycle.

One can not but recall in this connection another group of cases which give a history of uninterrupted menstruation throughout the entire period of pregnancy. In some of these cases it is evident that it is a question of more or less regular hemorrhage rather than of true menstruation, and it may be possible that in the others these hemorrhages happened to fall at intervals of the same length as the normal inter-menstrual periods. In the

last group of cases the exact status can not be discerned from the histories alone. However, since there is no endometrium or at most a partial one, to shed, genuine menstruation manifestly can not occur throughout pregnancy. It seems more probable that the cases of supposed uninterrupted menstruation fall into the last group although it is not impossible that rarely hemorrhage may occur only at the dates of normal recurrence. This would seem possible because of the defective inhibition of the returning normal impulses exercised by the dead or dying conceptus and the retrogressing corpus luteum. This conclusion harmonizes with the tendency to abortion which seems to be present at the time of periodic hyperemia and hyper-irritability in some of these cases.

Although dissolution of the embryo or fetus alone has long been known to occur, there seems to be no convincing evidence in the literature for the occurrence of intrauterine autolysis and absorption of entire conceptuses. Moreover, as already stated, it is to be doubted very seriously whether complete intrauterine absorption is possible after the formation of the skeleton has been well begun. The fetal parts at this time are so resistant that the uterus is stimulated to expel the macerated remnants long before absorption of them can occur. Furthermore, complete absorption would also seem to be hindered by the physical conditions which obtain in the human female. Only as long as the decidua capsularis is relatively thick, and hence effectively prevents the escape of the products of autolysized embryonic tissue, would complete absorption of a young uterine conceptus seem to be possible. The same thing probably is true in some cases of tubal pregnancy although the occurrence of early and repeated, yes, even prolonged, hemorrhage in them makes complete intra-tubal absorption much less likely. However, that complete intrauterine absorption actually does occur in the human female, it is the object of this article to establish.

The occurrence of missed abortion and also of missed labor long have been matters of common knowledge among physicians. But these are phenomena usually connected with the later months of pregnancy. In the overwhelming majority of these cases the fetus was retained for a considerable period after its

death and then aborted in a more or less macerated condition. Under these circumstances it may, to be sure, undergo absorption in part, but expulsion of macerated or even calcified remnants of the fetus nevertheless eventually occurs. That this is so even after an exceedingly long period of retention in utero, is shown splendidly by the case of Schaeffer, '98.

FIG. 1. External appearance of No. 698. The excised portion was used for microscopic examination. × 1.

Among the large series of over 2,000 abortuses in the Mall Collection, I have so far found only a few specimens of almost complete intrauterine absorption. In one of these, number 698, only a few vestiges of syncytium and trophoblast remain. Not a single fragment of the chorionic or amnionic vesicle or of the embryo could be found upon microscopic examination. Were it not for the presence of decidua and the above microscopic embryonic remnants, one might doubt whether pregnancy really had supervened in this case. However, since the specimen shown in cross section in Fig. 1 was aborted with the entire intact decidua which still surrounded the remnants of the conceptus completely as shown in Fig. 2 there manifestly could have been no loss of embryonic tissue either before or during abortion. This abortus, which measured 50 x 20 x 13 mm. was donated by Dr. N. E. B. Iglehart, of Baltimore. It had a menstrual age of 56 days, but the condition of the few remnants and the size of the implantation cavity show that development did not proceed very far before growth was inhibited. Aside from the absorption of almost the entire conceptus, the decidua not only is infiltrated, but also shows degenerative changes. As illustrated by Fig. 2, which shows the intact capsularis separated by a narrow space from the vera, the former is filled completely by blood clot. It is at the periphery of this clot that the isolated microscopic remnants of the syncytium and trophoblast, together with a few gossamer or shadow villi are found. Since

there is no blood between the capsularis and the vera it would seem to follow that the hemorrhage was limited entirely by the capsularis, a conclusion which is indicated also by the absence of a history of bleeding. Since the last menstruation began on April 11 and the abortion occurred on June 6, it is seen that the

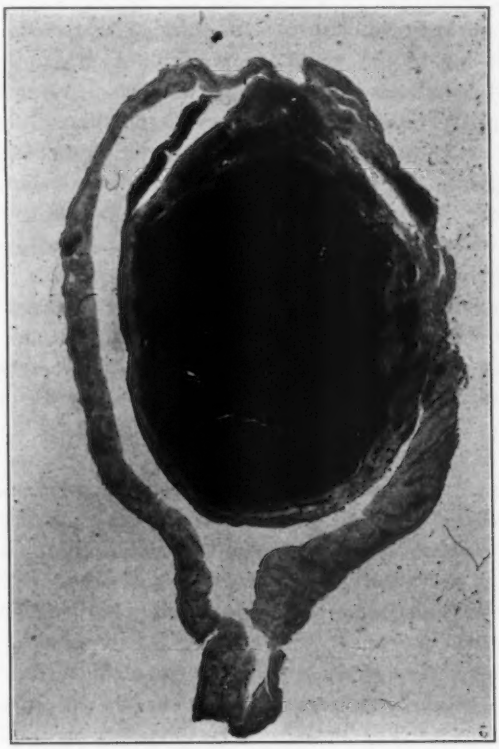

FIG. 2. Cross section of the above showing the disposition of the decidua and the contained blood clot. × 5.

latter occurred on the first day of the beginning of the second lapsed menstrual period. Although the appearance and the condition of the decidua seems to suggest that considerable regeneration of the endometrium had occurred, it is possible though unlikely, that the bleeding accompanying the abortion was

menstrual and that hence the abortion should be regarded as a mere incident accompanying the return of normal menstruation rather than as the predominating event.

That considerable restoration of the endometrium may occur while the conceptus still is within the uterus was shown by the case of Orloff, '95. In this case the endometrium was composed of a cylindrical epithelium and the uterine musculature showed no evidences of the presence of gestation changes. Ivanoff, '98, also found the decidua absent in a case of long retention and its place taken by a low cylindrical epithelium although the placenta still was partly attached to the uterus. Frankel, '03, also emphasized the fact that regeneration of the endometrium may begin before abortion occurs and these things make it possible that hemorrhage which may occur at time of abortion may largely be true menstrual hemorrhage.

The absence of blood between the capsularis and the villi and the absence of a history of bleeding do not, to be sure, imply that the development of this conceptus progressed uninterruptedly until birth. The histologic picture alone is conclusive proof to the contrary. In the absence of any larger portion of the conceptus it is impossible to say about how far development had proceeded, but it is unlikely that it proceeded much beyond the first month. In any case, disintegration, solution and resorption of almost the entire conceptus surely must have consumed several weeks at least. Indeed, it is possible that the ovum never became firmly attached, though imbedded in the decidua.

In other cases it also seems likely that the fertilized ovum became inbedded quite normally but that it was strangulated by severe hemorrhage which loosened the attaching villi, thus interrupting the intervillous circulation. Since the resulting stagnation of the blood must make the indispensable chemical interchanges upon which the life of the embryo depends, impossible, the latter probably dies first. It is decidedly interesting that considerable hemorrhage, sufficient, in fact, to result in the death of both the embryo and the chorionic vesicle, can occur while the whole conceptus still is surrounded by the decidua capsularis, without rupture of the latter. The failure of absolute

or complete absorption of the last few small remnants of this conceptus probably may be attributed to the fact that the small remnants of degenerate trophoblast and syncytium which remain, or the influence of the corpus luteum no longer were able to inhibit menstruation. Hence the decidua, together with these few small remnants of the conceptus were expelled *in toto* and it may be extremely significant that this occurred exactly two menstrual months after the beginning of the last period. Since three other specimens of a series of seventeen composed of villi only were aborted at the time of recurrence of the regular period, the idea that abortion occurs oftener at this than at any other time would seem to receive some confirmation. Moreover, it would seem quite natural that a detached decidua which has subserved its functions would be more likely to be shed at this time and that an unabsorbed conceptus which had been converted essentially into a foreign body, should then also be expelled. Since detachment of the decidua also permits regeneration of the mucosa and isolates the conceptus, it removes the inhibitory effects of the conceptus upon the maternal organism and clears the way for a return to the normal non-pregnant status.

It, to be sure, is impossible to decide how far the development of this conceptus had progressed before its death, but the marked extent of the absorption shows beyond doubt that the latter would have been completed long before the advent of the next or third menstrual period had the second period also been inhibited. Under these circumstances the empty decidual cast then would have been expelled alone and well might have directed attention to the possibility of the existence of a tubal rather than a uterine pregnancy.

A second case is number 970, donated by Dr. R. W. Hammack, of Manila. This specimen is interesting not only because it also is a case of marked intrauterine absorption, but because it was obtained with the entire uterus, at necropsy. The chorionic vesicle, which measured only 3 x 5 mm. together with the entire thickness of the decidua and the musculature, is shown in cross section in Fig. 3. The uterine cavity contained some blood and the entire decidua was covered with hemorrhagic nodules the

largest of which were about 10 mm. in diameter. One of these which was a trifle larger than the rest contained the conceptus. The cavity of the chorionic vesicle was filled with a homogeneous substance containing degenerate cells and portions of disintegrated chorionic membrane. The villi are only about .5 mm. in length and are covered with trophoblast and syncytial buds.

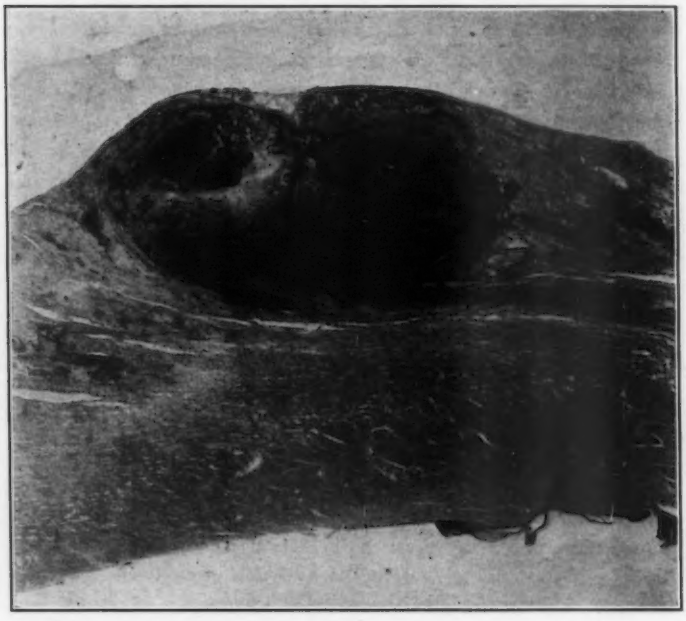

FIG. 3. Sections through the decidua and conceptus No. 970, showing a slightly bulging but intact capsularis.

No trace of the embryo or of the amnion was found, although the whole conceptus is covered still by the capsularis, which, like the rest of the decidua, is infiltrated. Although lysis and absorption did not progress so far in this as in the previous specimen, the process nevertheless is far advanced. It proceeded no further probably because the mother committed suicide, although the multiple hemorrhages in the decidua would seem to suggest that abortion nevertheless was seriously threatened,

if not inevitable, before she took her life. That the focal hemorrhages in the decidua could be attributed to the hydrochloric acid swallowed with suicidal intent, is extremely doubtful, for the histologic condition of the conceptus shows conclusively that the changes in it could not possibly have been produced in the short interval of four days which elapsed between the suicidal attempt and death. Since the menstrual history of the case remains unknown it is impossible to determine the menstrual age of the specimen but the degenerate chorionic vesicle would seem to imply an age of only about ten or twelve days. However as this young woman was but sixteen years and apparently illegitimately pregnant, it is more than likely that the suicidal attempt occurred, as so often is the case, during the time of the first lapsed period. Hence the surmise that the multiple hemorrhages in the decidua may have been provoked by the returning menstrual cycle, gains somewhat in probability. Especially so since the size and the condition of the conceptus both suggested that it must have died some weeks previous to its abortion.

A third early specimen illustrating the progress of intrauterine absorption is number 962, donated by Dr. Joseph M. Jackson, of Pittsburgh. In contrast to the preceding two, this chorionic vesicle contained a macerated embryo 4 mm. long. The menstrual age is unknown but the chorionic vesicle measured 34 x 28 x 24 mm. and was covered almost entirely by villi. The latter which contained degenerating vessels, are matted together with necrotic trophoblast and show other evidences of retention. As shown in Fig. 4, which represents a cross section of the entire conceptus with the surrounding decidua, the amnion was preserved and contained some coagulum. Mall found the embryo greatly macerated and the organs and cavities partially obliterated. The slight break in the decidua capsularis may be the result of handling or of technical procedures. Since the specimen was aborted with the entire decidua there can be no question of escape of a portion of the conceptus.

That it not alone is very young conceptuses which may undergo almost complete lysis, is illustrated by number 606, a chorionic vesicle measuring 18 x 13 x 18 mm. This specimen, which was donated by Dr. Charles S. Parker, of Baltimore, is covered with

villi 2.35 mm. long. Yet Mall found no trace of an embryo and stated that the villi and the chorionic membrane are structureless in spite of the outwardly normal appearance of the chorionic vesicle. In the absence of the clinical history one must needs be cautious but I think it safely can be assumed that in this case neither embryo nor amnion disappeared solely as a result of postpartum maceration. That this assumption probably is

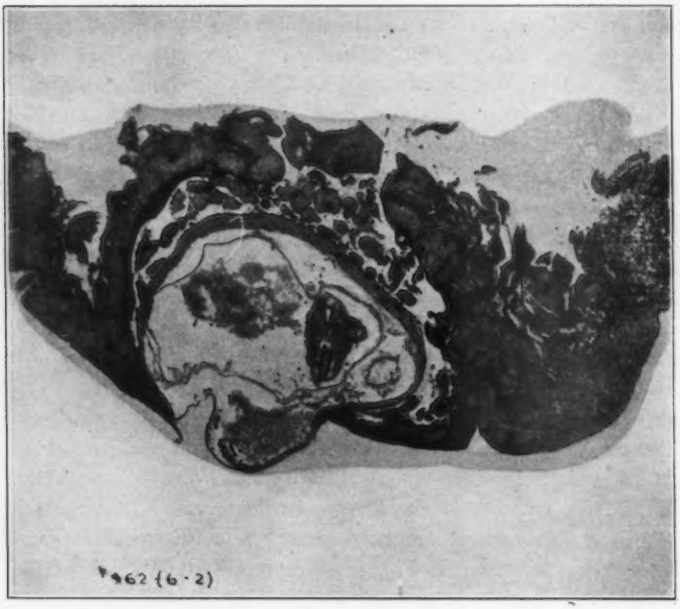

FIG. 4. Section through the decidua and the contained conceptus No. 962. The capsularis is slightly broken.

correct is shown also by other specimens, the histories of which fortunately are known. Nevertheless, maceration, although not necessarily putrefactive maceration, undoubtedly was an important factor in the production of the state in which this specimen is found. This conclusion is confirmed by the occurrence of all manner of transitions between the almost perfectly preserved structure and the pure shadow or gossamer pictures

such as presented in the photograph of the cross sections of the villi of this specimen shown in Fig. 5. All that remains of the villi is a spiderlike web, the fibres of which are exceedingly fine but which nevertheless preserves the form of the villi and of the chorionic membrane so perfectly that Mall especially recorded that the external appearance of the chorionic vesicle was normal.

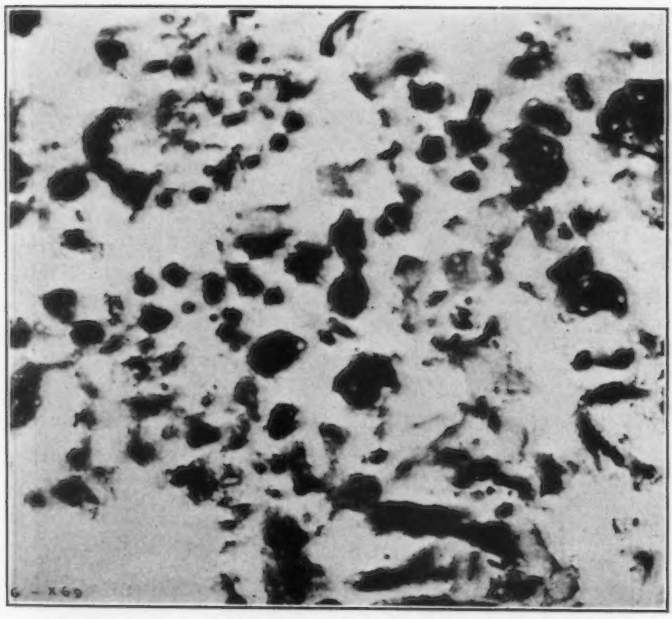

FIG. 5. Cross-sections of the gossamer villi of No. 606. × 69.

Since chorionic vesicles devoid of an embryo when examined form about 32 per cent. of those classed as pathologic in the Mall Collection it is evident that absence of the embryo itself is relatively common in early abortuses. It would be incorrect, however, to assume that they had undergone absorption in all these cases. A fine example of one of these empty chorionic vesicles is number 1224, a portion of which is shown in Fig. 6. This specimen was found in an unopened uterus removed at hysterectomy for cervical myoma. The chorionic vesicle,

which measured 36 x 25 x 13 mm. was collapsed, free from the uterus, and imbedded in coagulum. The only content of this chorionic vesicle was a dark grayish coagulum which contained no remnant of the embryo or of the amnion. This almost amorphous so-called magma included only a few isolated cells. In

FIG. 6. Section through the isolated empty conceptus and the adjacent decidua of No. 1224.

spite of this fact the trophoblast, which had markedly proliferated, was well preserved over large areas, and many of the vessels in the chorionic membrane were filled completely with erythroblasts. A few degenerate masses of trophoblasts and fused degenerate villi also are present. Some villi show evidences of

maceration, others of "mucoid" degeneration although they still may contain vessels. Some, however, are represented by a hyaline outline only. The stroma and the epithelium of many of the villi are well preserved, however, and the same thing holds for the chorionic membrane. The decidua shows slight general and very marked local infiltration. Some remarkably dense periglandular and perivascular zones of infiltration also are

FIG. 7. External appearane of No. 1843. × 6.

present. The mucosa, too, is infiltrated and contains islands composed exclusively of round cells. Besides maceration effects, many of the villi show marked changes, undoubtedly hydatiform in character. In this case it is possible that we are dealing not so much with absorption as with dissolution of the embryo, for the digestion products of embryo, yolk sac and amnion, instead of having been wholly absorbed still may be contained in the fluid within the chorionic vesicle.

Before briefly considering the evidence regarding absorption offered by tubal and ovarian specimens, I wish to refer to number 1843. This specimen, which was donated to Stanford Uni-

versity by Dr. Eugene V. Falk, of Modesto, California, had the villi rather sparsely and irregularly distributed as Fig. 7 shows. However, the entire specimen was so splendidly preserved that investigators of unique opportunity and experience were uncertain as to its normality. Even after careful inspection under low magnification the writer, too, felt uncertain, but on receipt of the specimen he had informed Dr. Falk that it probably was pathologic. This opinion was based almost wholly on the irregular distribution of villi, their complete absence on part of the surface; on the large size of the yolk sac; the unusual translucence of the entire specimen; and upon the *apparent* absence of the embryonic disc. About one-third of the entire surface of the chorionic vesicle was devoid of villi and where they were present they seemed to be in widely different stages of development. They differ markedly not only in length and in diameter but also in the complexity of their branching. Some which were represented by fine threads merely were found to be represented by stroma only, the epithelium having been stripped, probably during the removal of the blood in which the specimen was imbedded before it was received at the laboratory. Other villi are torn, those which are preserved are clubbed but slightly and some are so short that they look like little droplets on the surface of the chorionic membrane. Those near the bare areas are almost transparent but nearer the other pole they become more opaque. The caliber varies from 1/6 to 1 mm. and the greatest length is 2.25 mm. The chorionic vesicle measured 6 x 4 x 5 mm. and the partially invaginated yolk sac, 2 x 2.6 x 1.8 mm. A smaller, less transparent vesicle, which was thought to represent the amnion, was seen between the yolk sac and the chorionic membrane, but no embryonic disc could be recognized. An examination of the microscopic sections of this vesicle showed that it was macerated. The epithelium is missing in many places and the histologic details are wanting. Hence this chorionic vesicle very evidently ceased to live some time before it was aborted, and this conclusion is corroberated also by the clinical history. The menstrual age of the specimen is 39 days but the chorionic vesicle, which was approximately spherical, measured only 6.5 mm. instead of 25 mm. to 30 mm. as implied

by the menstrual history. That growth ceased long before the occurrence of the abortion is implied also by the fact that Dr. Falk stated that the patient had a slight uterine hemorrhage about 10 or 12 days before the abortion. It is interesting, and probably also significant that the date of this hemorrhage also coincides with the time when the first lapsed period was due. This is possible because this conceptus apparently had been dead sufficiently long to fail to inhibit the return of menstrual bleeding. Since the size of the chorionic vesicle suggests an age of 10 to 12 days, this assumption seems warranted, especially since it may be assumed that development probably never proceeds undisturbed to the time of abortion, whenever the pregnancy is terminated spontaneously or perhaps better, without the intervention of external or of internal mechanical forces or factors It may be largely because of this fact that conceptuses from abortions resulting from intrauterine causes always are macerated.

Careful examination of the serial sections generously made in the Carnegie Laboratory of Embryology, fails to reveal any remnant of the body of the embryo except perhaps a small nodule shown above (2) in Fig. 8. Yet according to the menstrual age the embryo should be 10–12 mm. long. The appearance of this nodule suggests that it may be a remnant of the primitive streak in spite of its deep location, although it may also be a rudiment of the allantois. The yolk sac is large and invaginated and its size out of all proportion to that of the embryonic Anlage. Between the latter and the chorion there is a rather large mass of cells containing a space which I take it, represents the amniotic cavity. An examination of this cavity and of the surrounding cells suggests that it resulted from splitting as is the case in bats and as is assumed for man. The cavity, to be sure, may have resulted from dissolution of the cells *in situ* and if in fact it represents the early amniotic cavity, then it is certain that whatever its genesis, it was not formed in consequence of folding. It would seem then that we have here a conceptus in which the process of development of the embryo itself was inhibited very early and that the yolk sac and the chorion continued to grow for some time.

Aside from showing a probable and hitherto unobserved stage

in the formation of the amniotic cavity, this pathologic chorionic vesicle is of special interest also in the absence of coagulum or so-called magma. The chorionic vesicle and the space taken for the amniotic cavity were filled with perfectly clear fluid. Although this small conceptus is markedly macerated it is

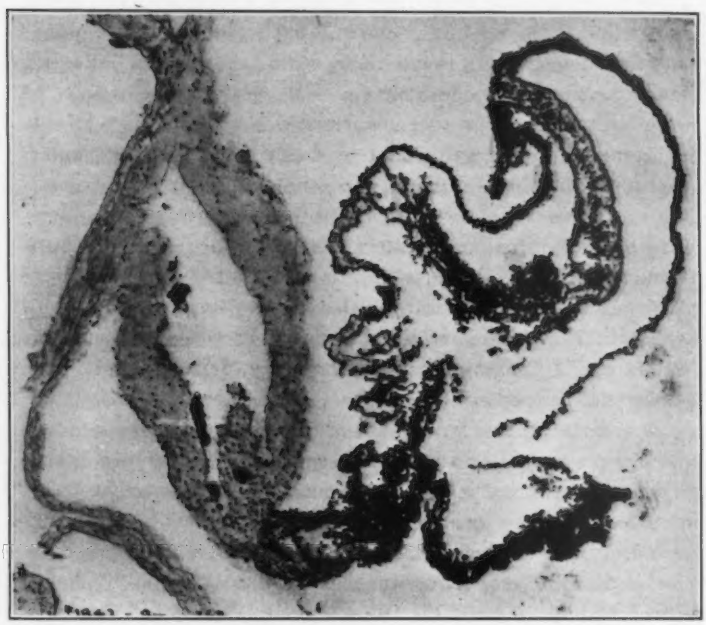

FIG. 8. Section through the chorion, amnion (?) and yolk sac of No. 1843 at the point of attachment. The small nodule of cells above (2) may be a remnant of the primitive streak or of the allantoic stalk. × 69.

only in the early stages of disintegration. Nevertheless, much longer retention might have resulted in its complete dissolution even if not its complete absorption. Since this small vesicle was aborted in its entirety with blood clot, it is highly probable that most of these degenerative changes occurred after it was loosened from the implantation site, probably by rupture of the decidua capsularis in consequence of hemorrhage.

In other older conceptuses in earlier stages of disintegration,

as number 2047, the amnion is preserved and distended as shown in Fig. 9. Both chorion and amnion are macerated and distended with clear fluid and it seems strange indeed that the embryo and the yolk sac may disappear completely without even a final clouding of the amniotic fluid. There may have been temporary clouding, but every now and then a specimen is received in which both the vesicles are distended with absolutely clear

FIG. 9. Gross appearance of abortus No. 2047 showing a large blood clot containing a transparent chorionic and an empty amniotic vesicle. X 3.

fluid, a fact which also implies that autolysis of the embryo occurred without digestion of the enveloping amnion.

Since only a pseudo-decidua forms, and then but rarely and very early, in cases of tubal pregnancy, the sequence of events leading to dissolution of the conceptus must be rather different. There can be no accumulation of blood in a capsularis. Hence the conceptus usually becomes isolated in blood or blood clot within the tube, and undergoes degeneration as the hemorrhage

continues and the tube becomes distended. If detachment of the conceptus occurs very early, it is conceivable that it may undergo complete disintegration also within the tube, that the latter may heal and the symptoms subside completely. While the occurrences of such instances of spontaneous cure of tubal pregnancy undoubtedly are exceedingly rare, evidence at hand seems to show that they can not be wholly excluded. This, I am told, agrees also with contemporary clinical opinion.

The possibility of tubal abortion with subsequent intraperitoneal disintegration, lysis and absorption must, to be sure, also be borne in mind. The occurrence of nothing but fragments of villi in a tubal clot as shown in the case of number 977 represented in Figure 10 cannot be accepted as positive proof that all the rest of the conceptus was absorbed intratubally. A portion may have been aborted, yet specimens such as number 2035 and 1938 speak eloquently for the *possibility* of absorption. The embryo and yolk sac in the latter specimen are disintegrated almost completely and the scarcely recognizable remnants lie isolated in the chorionic cavity which is moderately filled with an amorphous coagulum. The stroma of the chorionic membrane is œdematous and degenerate but nevertheless contains some well-preserved vessels, a few of which contain some blood cells. The same thing is true of the stroma and of the vessels of the villi which also are in process of dissolution. A moderate amount of trophoblast is present but there is very little syncytium. The epithelium of some of the villi has undergone hyline degeneration. The blood cells in the large clot in which this chorionic vesicle, which measured 8 x 5 mm. in section, was imbedded, are preserved fairly well, especially near the vesicle. Nevertheless, the old conceptus very apparently is in a state of disintegration and lysis, the tube wall is very thin and the mucosa congested, hemorrhagic and atrophic.

Number 2035, also a tubal specimen, likewise is an empty chorionic vesicle in process of disintegration, and many other specimens might be listed, but these examples suffice to indicate that intratubal, as well as intrauterine lysis and in part at least of absorption, of conceptuses undoubtedly occurs in the human being. It is of course exceedingly unlikely that in case of the

uterus this can occur before the impregnated ovum is imbedded, for failing to imbed it undoubtedly would escape relatively promptly although the observations of Kirkham, '16, show that the fertilized ova in mice suckling their young, may lie unimplanted within the uterus for over a week.

A free fertilized or unfertilized ovum which disintegrated within the tube on the other hand, might be absorbed completely. The same thing holds for early conceptuses within the

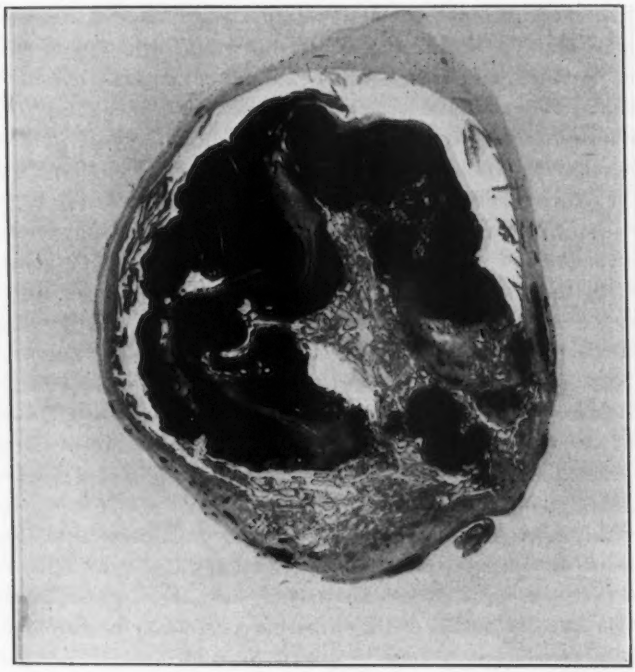

FIG. 10. Cross section of a tube with the contained blood clot and scattered chorionic villi, No. 977. × 4.4.

implantation cavity, for a relatively small amount of hemorrhage could detach them completely without rupturing the capsularis. Death, disintegration, and absorption, as illustrated in the cases above, then might occur. In case of older specimens the hemor-

rhage responsible for the loosening of the conceptus would have to be proportionately much greater for the attachment of the placenta is firmer though rupture of the capsularis easier. That this assumption is correct is shown also by the almost universal history of bleeding in these cases and it is only in the early stages of development that the conceptus can be expelled entire while still contained in the implantation cavity, and aborted completely wrapped in the decidua as in case of number 698.

Since 12.8 per cent. of all abortions and 32.3 per cent. of all those classed as pathologic in the first one thousand accessions in the Mall Collection are composed of villi only, of empty chorionic vesicles, and essentially of chorion and amnion, one might assume that all these specimens represent stages in the process of intrauterine disintegration and absorption. Most of them undoubtedly do belong in this category but in many cases in which villi only are found the rest of the conceptus and in others the embryo as well, has been lost. Since 46.4 per cent. of all tubal specimens and 71. per cent. of all those classed as pathologic are composed of villi only, of empty chorionic vesicles and of chorion and amnion only, it might be assumed that digestion and absorption are more active within the tube than the uterus. Such a conclusion is not justified however, for almost all tubal specimens are isolated while young and only exceptionally does one reach the later months of pregnancy. Hence those falling in the above-named three groups of tubal specimens form a relatively larger percentage.

Cases of partial dissolution of the embryo are of course common, as almost every one knows. As far as I can learn however, the two cases reported here are the only ones offering unequivocal proof that dissolution of the entire conceptus may be absolutely complete and that the intact empty decidua then may be aborted. Such an event could for various reasons probably occur only in the early stages of pregnancy and must undoubtedly be relatively rare. Nevertheless, I am convinced that careful examination of all material aborted will multiply the evidence. From these things it also would seem possible that rarely pregnancy may supervene and be terminated without having attracted any attention whatsoever.

The phenomenon of intrauterine lysis is interesting also from a chemical standpoint. What the antomist would like to know is not merely in what respects the composition of the intra- and peri-amniotic fluids has been changed, but just what the enzymes are that have caused a complete lysis of the embryo; where these first arise and act; and how and why they become active. These, and many other questions the chemist only can answer. For this answer fresh material is indispensable, but this the neighboring practitioners or a closely associated clinic can supply. That the lysis of these early embryos and undoubtedly also of the chorionic vesicles is not due primarily or even very materially to phagocytic activity is very evident even upon cursory examination. In the presence of the intact chorionic and amnionic vesicles in some specimens, such a process is wholly excluded. Besides, one never sees any evidence of phagocytosis of the preserved fetal by the maternal tissue in human conceptuses. Evidences of the contrary process are not wanting.

That the embryo or fetus usually is the first member of the conceptus to disappear has already been implied by stating that in 32 per cent. of the abortuses grouped as pathological in the Mall Collection, the embryo is missing. Although the absence of the embryo does not necessarily mean, in every case, that it underwent complete autolysis, this no doubt is true in by far the great majority of these cases. The fact that the embryo disappears first may be due to a lower resistance on its part than that possessed by either amnion or chorion, or to a preponderance of enzymes within it. Autolysis of the body of the embryo before that of the membranes, also may be due to the fact that the adnexa, especially the chorion, or at least parts of it, often preserve vitality long after the death of the embryo because of the direct relation to the uterus. Nevertheless, it often is surprising how long the form of a small retained fetus or even of the amnion apparently has been preserved, although it should be remembered that in some cases the preservation of external form gives little indication of the true state of the constituent tissues. If the advent of proteolytic fat-, and carbohydrate-splitting enzymes in fetal tissues is gradual, the surprisingly long time during which some of the small embryos are preserved may be due in a con-

siderable degree to this fact. Jones and Austrian, '07, found, for example, that the livers of young pig embryos contain no nulein enzymes, but that these increase as fetal development proceeds. The latter finding seems to be confirmed also by data given by Mendel and Leavenworth, '08, although these investigators found enzymes present at all ages. Schlesinger, '03, who was, I believe, the first to establish the occurrence of autolysis in retained human fetuses, also found that it varied with age and other conditions.

Since the chorionic villi do not undergo autolysis first, the conclusion that heterolysis is not an important factor in lysis of the conceptus, also seems justified. If enzymes of decidual origin played any large part in the process of lysis of the conceptus, one might, I presume, expect to find instances in which at least portions of the chorionic vesicle underwent lysis although the embryo itself remained quite unaffected. This, however, never seems to be the case and the same thing holds for the placenta and the chorionic vesicle and also for the decidua. That heterolysis probably plays only a very subsidiary rôle is indicated also by the fact that autolysis takes place in tubal implantations as well, and still more convincingly by its occurrence in ovarian implantations, as illustrated especially by the case of Holland, '11, and by other cases referred to by Meyer, and Wynne '19, in which the escape of portions of the conceptus from the ovarian implantation cavity could be wholly excluded. One reason for prolonged preservation of the decidua no doubt lies in the fact that it as a whole or at least in large part retains vitality because it remains quite undisturbed in its vascular relations.

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